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JOURNAL OF THE

# American Geriatrics Society

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VOLUME IV *February, 1956* NUMBER 2

## LIPID METABOLISM AND THE EFFECT OF A COMBINED LIPOTROPIC AGENT* †

WILLIAM B. RAWLS, M.D.‡ AND JEROME B. TICHNER, M.D.§

*The Research Department, New York Polyclinic Medical School and Hospital, New York, N. Y.*

In any present-day study of the problem of lipid metabolism, several important questions immediately pose themselves:

1. Are isolated blood cholesterol determinations and phospholipid cholesterol ratios of any value?
2. In what way do lipotropic agents influence these factors?
3. Is there any relationship between the size of lipoprotein particles and atherosclerosis? If so
4. What is the particle size?
5. What is the effect of lipotropic agents upon certain lipoprotein particles, as determined by dark-field microscopy?

For purposes of simplicity, the present study was arranged in two parts:

Part I. An effort to ascertain if single blood cholesterol determinations and phospholipid-cholesterol ratios were valuable, if they varied in different diseases, and if they could be influenced by lipotropic agents.

Part II. A brief review of the literature on 1) the relationship, if any, between the size of lipoprotein particles and the development of atherosclerosis, 2) the absence of correlation between total cholesterol levels and particle size, and 3) the effect of a combined lipotropic agent upon the size of lipoprotein particles, as determined by dark-field microscopy.

### Part I

#### EFFECT OF LIPOTROPIC AGENTS ON BLOOD CHOLESTEROL AND PHOSPHOLIPID LEVELS

*Introduction*

In a series of 400 patients, Eiber *et al.* (1) found no significant change in cholesterol or phospholipid values in any age group, and considered C-14 labeled cholesterol studies to date to be inconclusive regarding the factors involved in the formation of atheromatous lesions.

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† Supported in part by a grant-in-aid from the Lipotropic Research Foundation of New York, and the U. S. Vitamin Corporation (Arlington-Funk Laboratories).

‡ Attending Physician.

§ Assistant Attending Physician.

Goldbloom and Eiber (2) concluded that the Pl-CH ratio[1] was not constant and that ratios below 1 could not be looked upon as suggestive of any overt vascular disturbance.

Morrison (3), in a series of 124 patients who had recently had coronary thrombosis and myocardial infarction, found that a Pl-CH ratio below 1 was the abnormal value found in the majority of patients with active coronary atherosclerosis, and that a Pl-CH ratio above 1 was found in most normal individuals. He further stated that in 28 patients suffering from coronary atherosclerosis who had a subnormal Pl-CH ratio, the ratio was restored to normal levels (above 1) by means of lipotropic agents and diet.

On the other hand, Goldbloom *et al.* (2), in a controlled study, found the Pl-CH ratio unaltered by lipotropic agents. They attached no particular importance to the Pl-CH ratio because of the wide variation in both normal and abnormal subjects.

The foregoing observations are examples of the many and conflicting points of view regarding the role of cholesterol in the production of atherosclerosis, and the importance of the Pl-CH ratio. Being fully aware of these conflicting views and of the varied reports of the value of lipotropic agents, it is hoped that this paper will clarify some of the debated points, rather than add confusion.

*Materials and methods*

The patients were divided into four groups—A, B, C and D.

*Group A* consisted of patients suffering from a variety of diseases upon whom single cholesterol estimations were carried out—many at the request of other physicians in a large general hospital.

*Group B.* In this group, weekly blood cholesterol measurements were made, totalling as many as 39 in some instances. Pl-CH ratios were determined during a period of six to seven months, using the conventional technique for the collection of suitable blood samples. (Attempts were being made to establish a pattern.)

*Group C* patients were treated with a combined lipotropic agent, Methischol[2], in fixed daily doses (6 to 9 capsules), after their blood cholesterol levels and Pl-CH ratios had been studied for months. The normal cholesterol value used was 200–250 mg. per 100 ml. (Bloor) and 1.0–2.5 was accepted as the normal Pl-CH ratio. At no time were dietary measures employed. Our aim was to determine the variations of blood CH levels and Pl-CH ratios by an accepted method in patients following a normal pattern of life and diet. In addition, this would help us to assess the value of lipotropic therapy without the introduction of diet as a complicating factor.

*Group D.* From noon, all the food these patients had was tea with sugar but no cream, and dry toast at dinner time. Specimens of blood were taken the next morning.

[1] Phospholipid-cholesterol ratio.

[2] Methischol, a product of U. S. Vitamin Corporation (Arlington-Funk Laboratories); 6 to 9 capsules contain methionine (0.66 to 1.0 Gm.), inositol (0.5 to 0.75 Gm.), choline dihydrogen citrate (1.6 to 2.5 Gm.), and vitamin $B_{12}$ (12 to 18 μgm.) plus desiccated liver and liver concentrate.

TABLE 1

| Disease | No. of Cases | Avg. Blood Chol. (mg./100 ml.) | Avg. Age (yrs.) |
|---|---|---|---|
| Lipoid dystrophies | 5 | 649 | 42 |
| Periph. vascular dis. | 22 | 252 | 54 |
| Gastro-intest. dis. | 22 | 246 | 55 |
| Diabetes | 18 | 284 | 52 |
| Hepatitis | 8 | 277 | 45 |
| Pancreatitis | 3 | 301 | 57 |
| Hypothyroidism | 7 | 232 | 58 |
| Primary anemia | 3 | 191 | 56 |
| Gallbladder dis. | 14 | 247 | 57 |
| Renal calculi | 2 | 318 | 52 |
| Nephrotic syndrome | 4 | 506 | 28 |
| Gouty diathesis | 4 | 227 | 49 |
| Arteriosclerosis and/or heart disease | 51 | 286 | 59 |
| Metastatic cancer | 10 | 220 | 62 |
| Rheumatoid arthritis | 36 | 252 | 55 |
| Osteoarthritis | 121 | 283 | 57 |
| Viral gastroenteritis | 4 | 325 | 57 |

The entire series consisted of 334 patients (Table 1). Their ages varied between 20 and 90 years, the majority being in the fourth to seventh decades. A total of 1,596 blood CH estimations and 679 Pl-CH ratio determinations were made.

*Results*

*Group A.* It was interesting to note the variation in the average serum cholesterol levels, in groups of patients with the same disease. In the groups with arteriosclerotic heart disease and with osteoarthritis, the average value was above normal. Four patients with a diagnosis of viral gastroenteritis also had a high average cholesterol level, and the 5 patients with nephrosis, not unexpectedly, had a rather high level. In the 18 patients with diabetes the concentration of blood cholesterol was only in the high normal range (Table 1).

*Group B.* Blood CH concentration and Pl-CH ratio determinations were performed frequently (every week or two) during a period of at least six months, and often as long as twelve months. In 2 cases, as many as 39 determinations were made.

The findings in Patient M.C., studied for a period of over twelve months, are listed in Table 2. During ten of these months, the patient received no lipotropic therapy. Blood CH figures showed a wide variation, from 232 to 314 mg. per 100 ml.; and the Pl-CH ratio varied between 1.07 and 2.07.

*Group C.* The results of two months of lipotropic therapy in Patient M.C., are listed in Table 2. The blood CH level showed a variation between 232 and 260 mg. per 100 ml., but was never below the level observed without medication. During the same interval, the Pl-CH ratio varied from 1.7 to 2.4—a change of 0.7, as compared to a change of 1.13 without medication.

It might be suggested that lipotropic therapy in Patient M.C. was not continued long enough. However, Table 3 shows the results obtained in a patient

TABLE 2

*Data on Patient M. C., showing no appreciable change with lipotropic therapy*
(Based on 30 cholesterol determinations over a period of 13 months)

| Date | Blood Chol. (mg./100 ml.) | Blood Ph.-lipids (mg./100 ml.) | Pl-CH Ratio | Dose of Lipotropic Agent |
|---|---|---|---|---|
| 1954 | | | | |
| Mar. 2 | 314 | | | |
| Apr. 20 | 250 | | | |
| June 1 | 232 | | | |
| July 20 | 296 | 522 | 1.07 | |
| Sept. 7 | 248 | 630 | 2.07 | |
| Oct. 26 | 264 | 596 | 2.2 | |
| 1955 | | | | |
| Jan. 27 | | | | 2 capsules t.i.d. |
| Feb. 8 | 232 | 400 | 1.7 | 3 capsules t.i.d. |
| Mar. 15 | 242 | 496 | 2.4 | 2 capsules t.i.d. |
| Apr. 20 | 260 | 496 | 1.5 | 2 capsules t.i.d. |

TABLE 3

*Data on Patient M. S., showing no change with lipotropic therapy over a period of 8½ months*

| Date | Blood Cholesterol (mg./100 ml.) | Blood Ph.-Lipids (mg./100 ml.) | Pl-CH Ratio | Dose of Lipotropic Agent |
|---|---|---|---|---|
| 1954 | | | | |
| July 20 | 279 | 596 | 2.2 | |
| July 27 | 258 | 464 | 1.7 | 2 capsules t.i.d. |
| Aug. 17 | 252 | 450 | 1.7 | 2 capsules t.i.d. |
| Sept. 7 | 240 | 400 | 1.6 | 2 capsules t.i.d. |
| Sept. 28 | 278 | 400 | 1.4 | 2 capsules t.i.d. |
| 1955 | | | | |
| Feb. 1 | 200 | 416 | 2.4 | 2 capsules t.i.d. |
| Feb. 8 | 240 | 400 | 1.6 | None |
| Mar. 1 | 260 | 400 | 1.5 | 2 capsules t.i.d. |
| Mar. 8 | 264 | 496 | 1.8 | None |
| Mar. 15 | 228 | 438 | 1.9 | 2 capsules t.i.d. |
| Apr. 5 | 288 | 496 | 1.6 | 2 capsules t.i.d. |

receiving lipotropic therapy for eight and a half months; again, there was no appreciable change.

It might also be suggested that the dose of Methischol (6 capsules daily) was insufficient. Table 4 shows the results in Patient S.C. with a dosage of 9 capsules daily for five months; the cholesterol concentration at one point actually reached a higher level than before therapy. In Patient M.M. (Table 5) the Pl-CH ratio was 2.3 at the start of therapy and 2.0 at the end, although at one point it dropped to 1.1. Tables 5 and 6 show 2 patients with blood cholesterol levels ranging from high normal to high; again, there was no significant change in the cholesterol levels.

TABLE 4

*Data on Patient S. C., showing no change in cholesterol and phospholipid levels with lipotropic therapy in high dosage over a period of 5 months. There was, however, a change in the Pl-CH ratio*

| Date | Blood Cholesterol (mg./100 ml.) | Blood Ph.-lipids (mg./100 ml.) | Pl-CH Ratio | Dose of Lipotropic Agent |
|---|---|---|---|---|
| 1954 | | | | |
| Mar. 16 | 250 | | | |
| Apr. 13 | 300 | | | |
| May 11 | 302 | 480 | 1.5 | |
| June 8 | 320 | 556 | 1.7 | 3 capsules t.i.d. |
| July 6 | 264 | 570 | 2.1 | 3 capsules t.i.d. |
| Aug. 10 | 296 | 624 | 2.2 | 3 capsules t.i.d. |
| Sept. 21 | 320 | 690 | 2.1 | 3 capsules t.i.d. |
| Oct. 26 | 324 | 700 | 2.1 | 3 capsules t.i.d. |
| 1955 | | | | |
| Jan. 11 | 270 | 600 | 2.2 | None |
| Feb. 15 | 320 | 496 | 1.6 | |
| Mar. 7 | 304 | 496 | 1.6 | |
| Apr. 19 | 364 | 596 | 1.6 | |

TABLE 5

*Data on Patient M. M., showing no change with lipotropic therapy**

| Date | Blood Cholesterol (mg./100 ml.) | Blood Ph.-lipids (mg./100 ml.) | Pl-CH Ratio | Dose of Lipotropic Agent |
|---|---|---|---|---|
| 1954 | | | | |
| June 22 | 262 | 520 | 1.9 | |
| Aug. 3 | 254 | 594 | 2.3 | |
| Aug. 17 | 314 | 450 | 1.1 | |
| Sept. 7 | 296 | 496 | 1.6 | |
| Sept. 21 | 280 | 660 | 2.3 | 3 capsules t.i.d. |
| Oct. 26 | 284 | 660 | 2.3 | 3 capsules t.i.d. |
| Nov. 16 | 284 | 530 | 1.1 | 3 capsules t.i.d. |
| Nov. 23 | 312 | 524 | 1.6 | None |
| 1955 | | | | |
| Jan. 11 | 372 | 596 | 1.6 | None |
| Jan. 25 | 242 | 496 | 2.0 | 3 capsules t.i.d. |
| Mar. 22 | 288 | 400 | 1.3 | None |
| Apr. 5 | 304 | 580 | 1.9 | |
| Apr. 19 | 252 | 400 | 1.6 | |

* The cholesterol level varied but was consistently in a high normal range and at times above normal. Nine estimations of total cholesterol were made between June 22 and Sept. 21, in order to establish a normal pattern.

Patient S.C. (Table 4) was studied for a period of over twelve months, with lipid determinations before and after a 5-month period of therapy. This case appeared to be an exception to the rule because the Pl-CH ratio, during lipotropic therapy, was higher than before or after therapy.

TABLE 6

*Data on Patient A. W., showing no change with lipotropic therapy**

| Date | Blood Cholesterol (mg./100 ml.) | Blood Ph.-lipids (mg./100 ml.) | Pl-CH Ratio | Dose of Lipotropic Agent |
|---|---|---|---|---|
| 1954 | | | | |
| Aug. 10 | 346 | 564 | 1.6 | |
| Sept. 14 | 272 | 464 | 1.3 | 2 capsules t.i.d. |
| Oct. 5 | 278 | 524 | 1.8 | 3 capsules t.i.d. |
| Nov. 9 | 400 | 496 | 1.2 | 3 capsules t.i.d. |
| Nov. 23 | 312 | 496 | 1.5 | None |
| Nov. 30 | 340 | 475 | 1.3 | |
| 1955 | | | | |
| Feb. 15 | 288 | 430 | 1.5 | |
| Mar. 1 | 296 | | | |
| Mar. 22 | 266 | 580 | 2.1 | 2 capsules t.i.d. |
| Apr. 5 | 360 | 496 | 1.3 | 2 capsules t.i.d. |
| Apr. 19 | 330 | 496 | 1.5 | None |

* The consistently elevated cholesterol levels without treatment showed wide variations.

*Comment*

As a result of these observations, it was concluded that single determinations of blood cholesterol concentration or the Pl-CH ratio are of no particular significance.

The level of blood cholesterol may vary by as much as 100 mg. per 100 ml. and Pl-CH ratios may vary from month to month. Nevertheless, if a single blood cholesterol value is over 300 mg. per 100 ml., further estimations will most likely show high normal or high levels. On the other hand, if a single blood cholesterol value is 200 mg. per 100 ml. or less, subsequent estimations will tend to be within normal limits. Since, as a rule, values do not vary more than 100 mg. above or below the original, a single determination may serve to indicate a trend. However, when the original value is between 240 and 260 mg. per 100 ml., and the blood specimen is obtained at a time when the cholesterol peak is high for that patient, the findings may fall within normal limits; but if the blood specimen be taken at a time when the cholesterol peak is low for that patient, then the values will most likely fall into the high normal or high group. For patients in this high borderline group a single blood cholesterol estimation will fail to show even a trend; several determinations are necessary.

It was further concluded that the lipotropic agent used, even in high dosage for long periods, had no appreciable effect upon the level of blood cholesterol or upon the Pl-CH ratio. Moreover, it would be difficult to evaluate any apparently significant results, because of the wide variation in these values.

Morrison, in discussing the effects of lipotropic therapy in atherosclerosis, stated that these agents may be effective if (a) used with a low-cholesterol, low-fat diet, (b) given in large doses, and (c) administered continuously for six months. He added that unless this regimen be followed strictly, the therapy will be failure.

In our series, no dietary regimen was followed because of the difficulty involved in getting patients to adhere to strict diets and because of our desire to determine whether lipotropic therapy alone would result in an appreciable change in the level of blood cholesterol and in the Pl-CH ratio.

*Group D.* Even though there was fat starvation for eighteen hours preceding the collection of blood, there was still wide variation in the week-to-week blood cholesterol levels and Pl-CH ratios.

## Part II

### RELATIONSHIP BETWEEN SIZE OF LIPOPROTEIN PARTICLES AND ATHEROSCLEROSIS

Because of our negative findings, we sought other methods which might yield more information regarding the effect of lipotropic agents upon lipid metabolism. It was decided to study the effect of lipotropic therapy upon certain lipoprotein particles, by means of the dark-field microscope.

The subject of the role of lipoprotein particles in the production of atherosclerosis is in a confused state. We, therefore, present a brief review of the literature and a report of our own findings, in an attempt to establish some rationale for the theory of the relationship between particle size and atherosclerosis.

*Review of the literature*

Gofman and his associates (4–8) helped to focus attention upon the relationship between atherosclerosis and lipoprotein-particle size as a result of their work with the ultracentrifuge. Glazier *et al.* (4) stated that these particles may be divided according to flotation rates, a factor related to particle size, and reported measurements of the serum concentrations of Standard $S_f$ 0–12, Standard $S_f$ 12–20, Standard $S_f$ 20–100, and Standard $S_f$ 100–400. (The $S_f$ rate refers to Svedbergs of flotation[3].)

Gofman *et al.* (5) in 1951 concluded that certain lipoproteins ($S_f$ 8–10 and $S_f$ 10–20) are involved in the production of human atherosclerosis and that serum lipid is transported in lipoprotein macromolecules of varying chemical composition and physical properties. They added that hypertensive patients with associated coronary artery disease show higher $S_f$ 10–20 levels than patients with uncomplicated hypertension. They found the $S_f$ 10–20 lipoprotein molecule to be present in higher concentrations in those diseases associated with a high incidence of atherosclerosis, nephrosis and myxedema.

In 1950, Gofman *et al.* (6) stated that lipoprotein molecules with a rate $S_f$ 75 or more included the well known chylomicrons and aggregates of much smaller dimensions. They added that this entire class was increased after a fatty meal and represented an alimentary lipemia. They felt there was no relation between lipoproteins classified as $S_f$ 75 or higher, and atherosclerosis. Jones *et al.* (7) reported that lipoproteins above $S_f$ 100 are influenced principally by total lipid ingestion.

[3] Svedberg originally described the ultracentrifuge as designed to apply a force infinitely greater than that of gravity. Under this force, lipoprotein molecules can be made to undergo flotation if they have a density less than that of the solution in which they are contained. An $S_f$ unit represents a flotation rate of $1 \times 10^{-13}$ cm/sec./dyne/Gm. in a sodium chloride solution of 1.063 Gm./ml. density at 26°C.

More recently Pierce (8) stated that particles with $S_f$ rates of 40,000 are similar to chylomicrons and that generally $S_f$ rates parallel molecular size and are inversely parallel to the hydrated density. They added that lipoprotein particles below $S_f$ 12 are found in all normal human beings, and that particles between $S_f$ 12 and 100 are found in varying concentrations in several diseases, particularly in atherosclerosis.

These views are typical of the opinions held regarding particle size and its relation to atherosclerosis. However, one must admit that determinations of particle size have opened a new approach, and many ramifications are now being investigated by various research workers.

Bragdon (9) found that hypercholesterolemic serum from a rabbit fed on cholesterol, when given intravenously to a control rabbit, would induce the experimental animal's arterial lesions in the recipient within five hours after injection. Furthermore, within forty-nine hours, these lesions were well developed. Sudanophilic material could be identified within the endothelial cells and in the intracellular matrix of the intima. He also reported that if this hypercholesterolemic plasma were centrifuged in the ultracentrifuge, a fraction formed in the top few millimeters. This fraction, emulsified in saline and injected into a normal rabbit, caused no arterial lesions; however, injection of the infra-natant material caused typical vascular lesions. This suggests that the lesions may be induced by specific macro-molecules.

It may be added that the recognition of different sizes among lipid particles is not new. Gulliver (10) described the molecular particles of the chyle in 1847 and Müller (11) called them haemokonien or blood dust in 1896. Edmunds (12), viewing them under dark-field illumination, called them "motes floating in the sunlight." Gage and Fish (13) and Frazer and Stewart (14) also added to the knowledge of their behavior and variations. The latter found no increase in the number of these particles after the ingestion of carbohydrate or protein, but a marked increase after a fatty meal. During starvation the particle counts reached a constant basic level.

Moreton (15) suggested that the gradual development of atherosclerosis is related to the alimentary chylomicrons, which are the result of the repeated ingestion of many fatty meals over a prolonged period of time in predisposed individuals. Gofman (6) demonstrated that all major lipid fractions in the blood are present as giant lipoprotein molecules of several types.

Hueper (16), using macromolecular substances (acacia, methyl cellulose, pectin and polyvinyl alcohol), demonstrated that they caused vascular-wall damage similar to atherosclerosis. He concluded that the important factor in the production of atherosclerosis and arteriosclerosis by macromolecular substances was molecular size and metabolic stability.

Bryant, Palmer and Joseph (17) showed that non-autoclaved pectin solutions contained large pectin molecules which were retained in the tissues, whereas autoclaved pectin of the same molecular weight as plasma proteins failed to accumulate in the tissue. Later Woerner (18), as well as Hueper, both using lipids in guinea pigs, showed that the range of particle size is important in their deposition.

Friedman *et al.* (19) reported that all absorbed cholesterol is transported by the intestinal lymphatics, and recently they further reported that at least 95 per cent of the dietary cholesterol in the lymph is transported in the chylomicron fraction. They stressed the fact that it is no longer correct to assume that the dietary cholesterol is identical with that manufactured by the animal itself. The former enters the blood bound up with other lipids in an insoluble particle, whereas the latter is a solute. The chylomicron cholesterol enters the blood and, unlike endogenous cholesterol, is probably acted upon by two separate processes, the first of which is concerned with the disappearance of plasma turbidity. Since this turbidity is dependent upon the presence of colloidal particles which range in size from submicroscopic to microscopic aggregates (chylomicra), plasma clearing is dependent upon the alteration of such aggregates and consequent solution of lipids, including cholesterol. Friedman states that Moreton (15), sensing that chylomicra contained cholesterol, emphasized that abnormal persistence of this aggregate in the blood was the essential cause of atherosclerosis in man. It is of interest that Becker, Meyer and Necheles (20), as well as Zinn and Griffith (21), have presented evidence which strongly suggests that there is an abnormally slow disappearance of such chylomicra, both with advancing age and in atherosclerosis. Block, Barker and Mann (22) also gave indirect and partial support to Moreton's concept, for they observed a reduction in the clearing mechanism of atherosclerotic individuals; such clearing is equivalent to the dissolution of large macro-molecular aggregates or chylomicra.

Engelberg (23) stated that a portion of the serum cholesterol (about 20 per cent) filters through the excised arterial wall, part is deposited on the arterial wall, and the rest remains in the vascular lumen since it cannot penetrate into the arterial wall. He believes that the smaller proteins pass through the vascular wall into the lymph, that the largest lipoprotein particles cannot enter the arterial wall, and that the groups of intermediate size are the ones caught intramurally.

The material injected by Hueper (16) induced the same type of lesion as that found in atherosclerosis, except that the deposits consisted of the injected materials and not lipids. *The particle size of these substances was similar to that of microscopically visible lipoprotein particles* (*chylomicrons*).

Zinn and Griffith (21) and Labecki (24) noted a correlation between elevated chylomicron levels and the incidence of myocardial infarction. Our main interest, however, is not to establish a relationship between a high chylomicron-lipomicron ratio and atherosclerosis, but to establish the effect of a certain combination of lipotropic agents upon the chylomicron level.

### *Materials and methods*

Simultaneous determinations were made of blood cholesterol concentrations and Pl-CH ratio, as well as chylomicron-lipomicron ratios. The technique for determining the chylomicron-lipomicron ratio is abstracted from the article by Labecki (24) and is as follows:

> The subject is instructed to have the last meal at lunchtime of the day preceding the test and to eat nothing in the evening but tea with a little sugar (no cream) and a piece or two of dry toast. On the morning of the test, the subject is not allowed to drink water or

smoke, since both can induce peristalsis and thus affect the absorption of any residue in the intestine. A 10-ml. venous blood specimen is collected.

Immediately after the blood has coagulated, the tube is rimmed carefully, and centrifuged for fifteen minutes at 2000–2500 r.p.m.; the serum is then transferred to a clean tube and re-centrifuged for ten minutes at the same speed. When performing microscopic examination of serum, it is important that the slides and cover slips be chemically clean in order to eliminate all possible artifacts. A capillary tube is used to transfer one small drop of serum to a clean dry slide. The serum may be deposited by blowing gently. The cover slip must be pressed down firmly, to insure removal of air bubbles and a consistent depth of material. After making sure that the microscope is clean and free of dust particles and grease, and that the dark-field condenser is properly centered, the slide is placed on the stage. A drop of immersion oil is used on the condenser, and a drop on the cover slip.

The microscope is first focused on a square which is distant from the counting square, the counting square being most conveniently the one just below and to the right of the central ruled area in the net micrometer. Particles showing Brownian movement are clearly defined. Once this square is in true focus, the vision is transferred to the counting square.

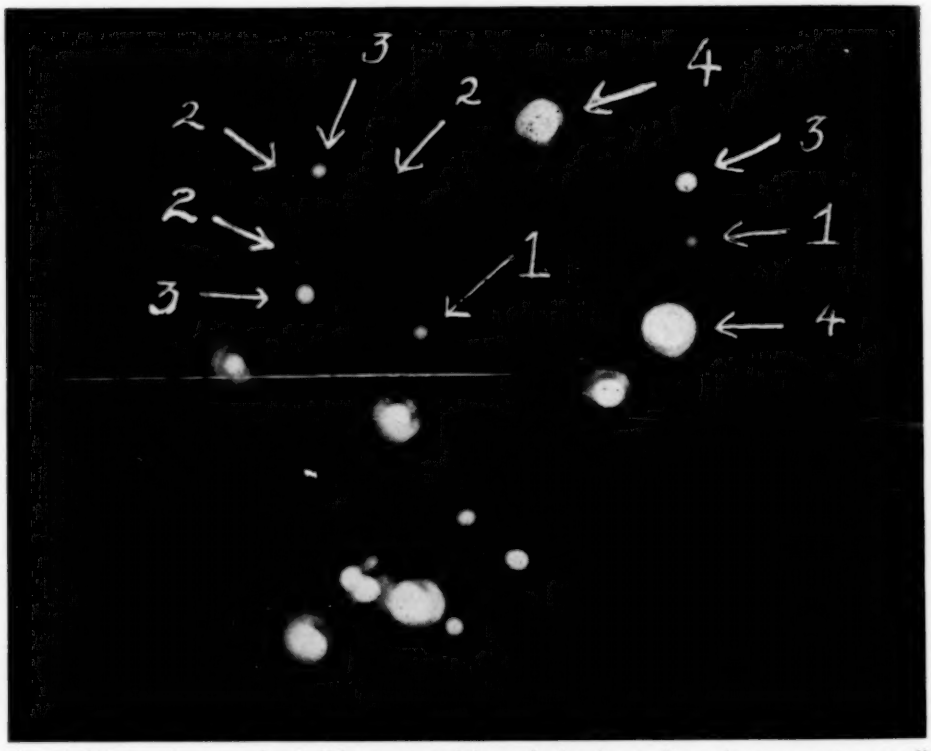


FIG. 1. Photomicrograph of slide prepared for achylomicron-lipomicron count according to the technique described.

The arrows indicate: #1, chylomicrons; #2, lipomicrons; #3, giant cells; #4, artefacts. The chylomicrons are larger and brighter than the lipomicrons but are smaller and not as bright as the giant cells. There is also some haziness around the periphery of the chylomicrons as compared to the giant cells. Under the dark-field microscope, the Brownian movement is easily seen and fairly rapid. The chylomicrons move in approximately a straight line, whereas the lipomicrons move in a zigzag course. The lipomicrons are smaller than the chylomicrons and not as bright. The giant cells are larger and definitely brighter than either the chylomicrons or lipomicrons. The artifacts are easily discernible under the microscope.

Some of the lipoprotein particles will appear larger and more brilliant than others which are poorly visible; the smallness and dullness of the latter are only infrequently due to the globules being in a deeper plane. The larger particles (chylomicrons) are approximately ⅓ micron or greater in diameter; the smaller particles cannot be measured accurately, but this is of minor importance. The number of chylomicrons and the number of visible particles per square are noted. Counting must be rapid, as Brownian movement causes particles to move from one square to another with considerable speed and the adjustment must not be changed while counting a pre-determined square. Ordinary double mechanical counters should be used. When one square has been counted, the stage is moved to bring another field into view and the counting procedure repeated until 200 squares have been counted. The concentration (expressed in per cent) is determined by dividing the total number of chylomicrons by the total number of visible particles and multiplying the value by 100. Counting is begun in the upper left corner of the cover slip; the entire area can be systematically covered.

For routine counts, a Spencer analytical binocular microscope is used, with a dark-field condenser, and oil immersion objective (1.8 mm. and a numerical aperture of 1.25), providing an initial magnification of 95×. With an appropriate eyepiece, the total magnification is about 2,000 diameters. In one of the oculars is inserted a net micrometer calibrated so that the distance between two fine lines is about 1 micron. A strong bulb in a regular microscope lamp provides adequate illumination. For particle size determination, the distance between two fine lines of the net micrometer is determined for any given combination of objective, eyepiece and tube. A Neubauer hemocytometer or a similar counting chamber may be used for this purpose.

Total and esterified cholesterol were determined by a modified Bloor's technique, using 1 ml. of serum made up to 25 ml. with Bloor's reagent. The aliquots were dried and extracted and the color developed by the original Lieberman-Burchard reaction, with careful attention to such factors as time and temperature. Leitz and Photovolt colorimeters were used for making the readings.

Figure 1 is a photomicrograph of the lipoprotein particles as they appear under the dark-field microscope.

*Results*

Sixty-six patients were studied. The chylomicron-lipomicron ratios were determined before and after therapy with the lipotropic agent (Methischol). In some instances, repeated checks were made on the same patient by first omitting the medication for varying periods of time, then allowing the ratio to return to its

TABLE 7

| Base No. | Normal Chol. (mg./100 ml.) | Normal C-L ratio (%) | Normal Chol. (mg./100 ml.) | High C-L Ratio (%) |
|---|---|---|---|---|
| 1 | 244 | 25.5 | 216 | 39.3 |
| 2 | 200 | 30.7 | 262 | 37.9 |
| 3 | 224 | 26.0 | 252 | 37.9 |
| 4 | 222 | 28.1 | 240 | 42.9 |
| 5 | 220 | 31.6 | 244 | 38.3 |
| 6 | 256 | 31.3 | 214 | 39.2 |
| 7 | 192 | 33.3 | 264 | 39.2 |
| 8 | 220 | 31.9 | 236 | 40.0 |
| 9 | 230 | 28.0 | 230 | 41.3 |
| 10 | 193 | 34.6 | 256 | 41.0 |

TABLE 8

| Case No. | High Chol. (mg./100 ml.) | High C-L ratio (%) | High Chol. (mg./100 ml.) | Normal C-L ratio (%) |
|---|---|---|---|---|
| 1 | 372 | 41.3 | 392 | 33.7 |
| 2 | 384 | 40.0 | 371 | 33.3 |
| 3 | 400 | 42.4 | 416 | 32.3 |
| 4 | 496 | 38.4 | 360 | 34.4 |
| 5 | 324 | 43.1 | 416 | 31.4 |
| 6 | 292 | 40.3 | 372 | 35.4 |
| 7 | 340 | 39.7 | 356 | 31.0 |
| 8 | 460 | 39.0 | 320 | 30.2 |
| 9 | 320 | 45.3 | 332 | 28.9 |
| 10 | 384 | 43.1 | 292 | 28.4 |

TABLE 9

| Case No. | Date | No. of Capsules | C-L Ratio (%) | Case No. | Date | No. of Capsules | C-L Ratio (%) |
|---|---|---|---|---|---|---|---|
| 1 | Nov. 1, '54 | 2 t.i.d. | 45.3 | 5 | Mar. 15, '55 | 2 t.i.d. | 41.0 |
| | Feb. 24, '55 | 3 t.i.d. | 31.0 | | Apr. 13, '55 | 2 t.i.d. | 32.0 |
| 2 | Nov. 4, '54 | 2 t.i.d. | 38.2 | 6 | Feb. 17, '55 | 3 t.i.d. | 39.2 |
| | Mar. 25, '55 | 2 t.i.d. | 27.7 | | Apr. 14, '55 | 3 t.i.d. | 29.2 |
| 3 | Dec. 23, '54 | 2 t.i.d. | 40.0 | 7 | Apr. 24, '55 | 2 t.i.d. | 44.6 |
| | Mar. 9, '55 | 2 t.i.d. | 33.9 | | May 4, '55 | 2 t.i.d. | 36.9 |
| 4 | Nov. 11, '54 | 2 t.i.d. | 41.3 | 8 | Mar. 1, '55 | 3 t.i.d. | 43.1 |
| | Mar. 31, '55 | 2 t.i.d. | 33.0 | | Mar. 15, '55 | 3 t.i.d. | 30.7 |

previous level, and finally re-instituting lipotropic therapy. When the lipotropic agent was omitted, there was a tendency for the ratio to return to pre-treatment levels and in a few instances, even above.

By means of lipotropic therapy, a reduction in the chylomicron-lipomicron ratio could be obtained almost at will. There were a few exceptions, 1 being a markedly alcoholic patient, and 1 a severe diabetic. In 1 overweight male (270 pounds), a dosage of 9 capsules of Methischol daily for four months was required to reduce the ratio to normal.

Table 7 shows a series of 10 patients with a normal blood cholesterol level and a normal chylomicron-lipomicron (C-L) ratio compared with 10 patients with a normal cholesterol level and a high C-L ratio.

Table 8 shows 10 patients with a high blood cholesterol level and high C-L compared with 10 patients with a high cholesterol level and a normal C-L ratio. These two tables illustrate that there is not necessarily any correlation between the blood cholesterol level and the chylomicron-lipomicron ratio.

Table 9 shows 8 patients with high C-L ratios, and the result of therapy with lipotropic agents. In every instance, there was a significant reduction of the C-L ratio.

TABLE 10

| Date | Dose/Time (capsules/days) | C-L Ratio (%) |
|---|---|---|
| *Case #1* | | |
| Nov. 30, '54 | Initial test | 40.3 |
| Jan. 4, '55 | 2 t.i.d./35 | 32.7 |
| Feb. 8, '55 | 3 t.i.d./35 | 28.3 |
| Mar. 1, '55 | None/21 | 49.0 |
| Mar. 22, '55 | 2 t.i.d./21 | 34.3 |
| Apr. 19, '55 | 3 t.i.d./27 | 34.2 |
| *Case #2* | | |
| Nov. 30, '54 | 2 t.i.d./21 | 39.7 |
| Feb. 15, '55 | 2 t.i.d./77 | 40.3 |
| Mar. 1, '55 | None/14 | 46.4 |
| Mar. 22, '55 | 3 t.i.d./21 | 33.5 |
| Apr. 5, '55 | 3 t.i.d./14 | 28.3 |
| Apr. 19, '55 | None/14 | 41.6 |
| May 17, '55 | 3 t.i.d./28 | 30.6 |
| *Case #3* | | |
| Nov. 23, '54 | Initial test | 47.0 |
| Dec. 26, '54 | 2 t.i.d./28 | 21.1 |
| Mar. 9, '55 | None/21 | 30.3 |
| May 4, '55 | None/28 | 46.1 |
| *Case #4* | | |
| Feb. 8, '55 | 2 t.i.d./28 | 26.3 |
| Feb. 15, '55 | None/14 | 31.6 |
| Mar. 15, '55 | None/28 | 40.2 |
| Mar. 29, '55 | 3 t.i.d./14 | 28.3 |
| Apr. 19, '55 | None/21 | 29.9 |
| *Case #5* | | |
| Feb. 15, '55 | 2 t.i.d./21 | 33.3 |
| Mar. 1, '55 | None/14 | 43.1 |
| Mar. 15, '55 | 3 t.i.d./14 | 30.7 |
| May 3, '55 | None/49 | 44.0 |

Table 10 shows the data on 5 patients in whom periods of lipotropic therapy were alternated with periods without therapy. In all instances, the C-L ratio was reduced by the lipotropic agents but in each instance there was a return to a high level when the medication was omitted, followed again by a decrease to normal when therapy was resumed. In Case #2 (Table 10) the initial dosage of 6 capsules of Methischol daily was not sufficient to reduce the C-L ratio but 9 capsules daily brought the ratio down to normal.

## DISCUSSION

We have shown in Part I of this paper that wide variations in blood cholesterol and phospholipids values, as well as in phospholipid cholesterol ratios, occur

from week to week or month to month. Moreover, there is no absolute relationship between the blood cholesterol or phospholipid levels or their ratios and the chylomicron-lipomicron counts. Labecki (25) states that his studies indicate a constancy of chylomicron-lipomicron counts, under normal conditions. We have further demonstrated that administration of a combination of lipotropic agents (choline, inositol and methionine) caused no significant changes in the blood cholesterol or phospholipids levels or in their ratio in either patients with known atherosclerosis or in those presenting no evidence of atherosclerosis. In contrast, a reduction in the number of chylomicrons could be obtained almost at will in most of the 66 patients in our series. This observation may be of some significance in the study of atherosclerosis. However, we should like to emphasize that the question of lipid metabolism and its role in the production of atherosclerosis is still unsolved. Morrison (26) states that despite the rapidly growing accumulation of information about lipid and lipoprotein metabolism we are still unaware of many of the fundamental mechanisms involved. Labecki believes that most likely no one fraction or factor entirely accounts for the development of atherosclerosis and that combined data on multiple fractions may yield highly significant correlations with the disease process. Friedman (19) states that many of the studies which were carried out by means of specialized apparatus have yielded data which, in the absence of proper physiologic perspective, have allowed concepts with "vertical extensions which are far greater than their bases will support." He further states that "perhaps in no other disease entity is there a greater need than in atherosclerosis for open-mindedness in the contemplation of its possible causes. Nevertheless, the impartial observer is constrained to conclude that such a state of mind is not always a common one in this field. Rather, it would seem that some individuals, or groups of individuals, deriving their impressions and drawing their conclusions from the limited data obtainable in their particular laboratories, endeavor to establish a single dominant cause for the occurrence of atherosclerosis, even at the cost of overlooking other data which militate against the application or even the essential verity of their particular generalization."

We are in full accord with these statements, but evidence is increasing that the size of lipoprotein particles may bear an important relationship to the development of atherosclerosis. However, there seems to be a growing difference of opinion as to the size of the particle involved. If this theory be correct—that the atherosclerotic process is set in motion by the deposition of lipoprotein particles of a certain size—then it is reasonable to assume, other factors being equal, that the higher the concentration of the offending lipoproteins, the greater will be the progress of the atherosclerosis; and, since coronary artery disease is largely dependent upon the presence of atherosclerosis, the greater will be the likelihood of coronary artery disease. If atherosclerosis be due to the concentration of lipoprotein particles of a specific size in the vessel walls, then any therapy that may reduce the number of these particles and maintain a normal ratio should tend to prevent the development of atherosclerosis in susceptible individuals.

Enos (27), however, demonstrated marked atheromatous changes in the arteries of combatants killed in the Korean war (mean age 21 years) and noted that the incidence and degree of change were associated with alterations in the anatomy of the coronary tree. The descriptions of the coronary vessels by Yater and associates (28), French and Dock (29), Moschowitz (30), and Moon and Rinehart (31) are in agreement that a diseased unoccluded coronary vessel does not show cholesterol deposits but does show a fibroblastic proliferation of the intima.

It is well established that whenever a blood vessel is traumatized by hypertension, vasospasm or any other cause, atherosclerosis is a frequent sequel, regardless of age, nutrition or blood cholesterol concentration. Therefore cholesterol may play only a secondary role; some form of trauma may be the exciting cause, and cholesterol infiltration may occur later. Friedman (19) states that in view of the recent discovery that all dietary cholesterol enters the blood enmeshed in chylomicra, this cholesterol not only differs in its physico-chemical characteristics from endogenously-produced cholesterol but must be handled, at least in part, by the body *i.e.*, by the hepatic reticulo-endothelial cells. He further states that, in view of this new data plus the fact that experimentally induced atherosclerosis depends upon the absorption of dietary cholesterol, it would perhaps be wise not to dismiss the findings of Moreton (15), Becker, Meyer and Necheles (20), Zinn and Griffith (21), and Block, Barker and Mann (22) or the concepts of Hueper (16) and Hirsch and Weishouse (32). The possible relationship of hyperchylomicronemia to atherosclerosis, although not proved, offers an opportunity for productive research.

## CONCLUSIONS

1. There is a wide variation in blood cholesterol and phospholipid levels from week to week.
2. Lipotropic agents, such as used in this study, have no appreciable effect on the blood cholesterol level or on the Pl-CH ratio, except in hypercholesteremia.
3. The chylomicron-lipomicron ratio is readily reduced in most instances by the use of lipotropic agents but, on omission of therapy, the ratio returns to its former level.
4. Although evidence is available which suggests that the particle size of lipoproteins may bear an important relationship to the development of atherosclerosis, this hypothesis has not been proved.

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# PHYTOPHARMACOLOGY AS AN AID TO DIAGNOSIS AND EVALUATION OF THERAPY IN PEMPHIGUS, PSYCHOSES AND CARCINOMA*

DAVID I. MACHT, M.D.

*Division of Pharmacology, Laboratories, Sinai Hospital, Baltimore, Maryland*

Thirty-two years ago the author, while working as an associate of Professor John J. Abel, the father of American pharmacology, at Johns Hopkins Medical School, conceived the idea of comparing animals with plants in respect to their responses to various drugs and chemicals. In order to do so, he spent more than a year in the laboratories of the late Professor Burton Livingston, head of the department of plant physiology at Johns Hopkins University (1). The methods for studying the effects of drugs on living vegetable test objects are numerous and cannot be described in detail here (2–4). However, just as certain standard animals are employed for learning the principles of physiology, so are the seedlings of the hardy plant, *Lupinus albus*, used extensively for teaching plant physiology. These seedlings were found to be most useful for our purposes because of their single straight roots when grown in plant physiologic solutions [Shive's (5)]. Thus a new department of biology was born, to which the name of *phytopharmacology* was given in contrast to *zoöpharmacology*, which is the ordinary pharmacology taught in medical schools. Numerous drugs and chemicals were examined and their effects on the root growth of Lupinus seedlings in hydroponic solutions under standard ecologic conditions were measured and statistically evaluated (6, 7). Some chemicals inhibited root growth as compared with normal controls, whereas others actually stimulated the growth of the plants (8). The results were usually expressed by what was termed the index of growth or phytotoxicity, as shown by the formula:

$$\text{Phytotoxic index} = \frac{X}{N} \times 100.$$

The most interesting result from such phytopharmacologic studies was the discovery of the phytotoxic properties of blood and various fluids from human beings and lower animals. It was found that the phytotoxicity of normal human blood serum was approximately the same as that of the serum of all higher and lower animals, with the exception of reptiles. With few exceptions, there was little variation in the phytotoxicity of sera from human beings, even in patients afflicted with all kinds of diseases, minor or major. The exceptions, because of their paucity, were of great interest not only from the diagnostic but also from the pathologic point of view, and were also useful in evaluating the progress of various diseases. A detailed description of such conditions is not appropriate

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here, but a brief mention should be made of the highlights in the development of this new science of phytopharmacology (9).

*Historical.* The first stage of phytopharmacologic studies may be termed historical. It led to the first experimental quantitative demonstration of the presence of a toxin (menotoxin) in the blood, saliva, sweat and secretions of menstruating women, thus confirming numerous popular beliefs on the subject among peoples ancient and modern (10–12). These studies also led to the discovery of a phytotoxic action on plant growth exerted by the blood from patients with true or Addisonian pernicious anemia (13, 14).

*Pemphigus and leprosy.* The second stage of phytopharmacology was a more important one from the clinical point of view. A well known dermatologist Dr. Isaac R. Pels of the Johns Hopkins Medical School came to the author expressing a wish to carry on an experimental research. This resulted in a phytopharmacologic examination in our laboratory of the blood in various skin diseases, and led to a striking and curious observation. With 2 exceptions, sera from every kind of dermatosis caused no more inhibition in the root growth of our seedlings than did normal human blood serum; and 1 skin disease, psoriasis, actually caused less inhibition in the root growth. The 2 phytotoxic diseases exhibiting characteristic poisonous effects on the seedlings were pemphigus (15) and leprosy. From the practical point of view, pemphigus was the more important, because leprosy patients were few and most of the work on their sera was performed on specimens sent from leprosy colonies and from India (16). The extensive researches by Pels and Macht on pemphigus, however, led to the establishment of the Macht-Pels test (17). This test is universally acknowledged to be diagnostic, except by a few "doubting Thomases" who criticized phytopharmacology without having had any experience along these lines. The writer still receives specimens of serum from various parts of the United States and occasionally from abroad, with requests for help in the differential diagnosis of pemphigus, which is especially difficult to diagnose in cases of lesions of the mouth and occasionally of the eyes (18). Not only has the diagnosis of pemphigus been aided by phytopharmacologic procedures, but also the treatment. Macht and Ostro (19) discovered the detoxifying or antidotal influence of certain filtered x-rays on pemphius serum *in vitro* and on the blood of pemphigus patients *in vivo*. Another gratifying achievement of the phytopharmacologic approach to the study of this highly fatal (90 per cent) disease was the author's finding that an admixture of minute quantities of suprarenal cortex extract actually resulted in a neutralizing effect on the toxicity of pemphigus serum (20). Later, similar *in vitro* experiments performed by the author with pure cortisone and with ACTH revealed a marked detoxifying effect on pemphigus toxin; and in blood sera from patients before and after initiation of adrenal corticosteroid therapy, a similar effect was demonstrated.

*Psychosis.* The third stage in the development of phytopharmacology may be dated from the time of the writer's announcement of his findings after ten years of cautious study of blood specimens from all types of psychotic patients (21).

The possibilities of the phytopharmacologic approach to the psychosomatic basis of mental disease were considered almost thirty years ago by Herz and Weichbrodt (22) in 1924 and actually tested with Macht's method by Tscherkes and Mangubi, who found that sera from psychotic patients had toxic effects (23). The writer carried on such studies seriously for ten years before he ventured to announce his results. Briefly, these findings revealed that the sera of all patients with true psychoses, whether so-called "organic" or "functional," exerted a phytotoxic effect on the root growth of *Lupinus albus* seedlings. The degree of toxicity varied, not with the diagnosis of a particularly labelled psychosis, but rather with the severity of the disease. These findings suggested the employment of the author's botanical approach to the evaluation of various therapeutic procedures in psychiatry. At the present time the writer and a well known psychiatrist are engaged in the study of blood sera from various mental patients. The specimens taken before treatment are compared with specimens taken after initiation of various therapeutic procedures, particularly treatment with the newly introduced drugs such as reserpine and chlorpromazine.

*Infections, psoriasis, syphilis, leukemia.* It is important to stress not only the valuable *positive* phytopharmacologic findings in cases of pemphigus and psychotic diseases, but also to call attention to the equally important *negative* findings in many other diseases. For instance, all bacterial infections yielded "nontoxic sera," which behaved for plants exactly like normal blood (24). Equally striking were the negative findings with the blood of psoriasis, syphilis, cancer and leukemia. The writer has considered the phytopharmacologic approach to these pathologic conditions for many years, and has conducted a systematic examination of various chemicals in regard to their influence on the growth of seedlings reared under normal conditions compared with that of "vernalized" seedlings from the same crop. Vernalization or, as the Russians term it, "yarovization" is a very important subject in plant physiology. It has been studied particularly by the Russians because of its bearing on agricultural problems (25, 26). The rationale of this phenomenon cannot be discussed in the present sketch, but the reader is referred to technical works. By experimenting with solutions of many chemicals and drugs, it was found that there was very little difference between normal and vernalized plants in their inhibitory effects on the root-growth of Lupinus seedlings. One chemical, however, was a striking exception. It was observed that ethyl carbamate or urethane exerted very little inhibition on the growth of normal seedlings, but markedly inhibited the growth of vernalized seedlings (27). This finding suggested a trial of the effects of various nontoxic blood sera on the two kinds of seedlings and led to an unexpected discovery, namely, that serum from psoriasis patients, although nontoxic for normal seedlings, is quite toxic for vernalized ones (28, 29). Similarly, the blood and cerebrospinal fluid of patients with syphilis was also toxic for vernalized seedlings, and the degree of toxicity ran parallel to the severity of the disease as indicated by the usual serologic data. This finding of a botanical test for syphilis was presented before the American Therapeutic Society and was published in its

Transactions (30). The foregoing statements should not be taken as a suggestion of any relationship between psoriasis and syphilis; however, with the exception of these two diseases and cancer, no other clinical condition showed a difference in phytopharmacologic effects on normal and vernalized seedlings. In fact the blood sera of patients with syphilis or psoriasis can be easily distinguished pharmacologically in the test tube (31). The writer and his assistants found that admixture of small quantities of penicillin or oxytetracycline markedly decrease the phytotoxicity of syphilitic blood but not of psoriatic blood; on the other hand a minute quantity of cortisone will detoxify the serum of psoriasis but will have no effect on the serum or spinal fluid of syphilis.

*Cancer.* As mentioned previously, blood sera from patients with bacterial diseases, syphilis, psoriasis, leukemia and cancer did not have any more toxic effects on root growth than did normal blood sera. Nevertheless, the sera of both psoriasis and syphilis when tested on vernalized seedlings did exhibit marked phytotoxicity. This fact, taken together with the well known observations of other investigators on the special effects of urethane in leukemia (32) and in certain inflammatory conditions (33), stimulated an inquiry into whether the sera from patients with cancer would affect vernalized seedlings. In a series of experiments it was found that blood sera from patients suffering from malignant tumors such as carcinoma, sarcoma, and leukemia had a phytotoxic action on the root growth of *Lupinus albus* seedlings, although sera from exactly the same patients when tested on normally-reared plants did not cause any inhibition in root growth. Furthermore, when blood specimens taken from patients with benign tumors such as myoma, lipoma, and benign polyps were tested on vernalized seedlings they did not cause any phytotoxic reaction. This investigation was carried on for two and a half years until a total of 300 patients with tumors were studied. These patients presented some 50 varieties of clinico-pathologic material. On these 300 patients a total of 1,200 serum determinations for phytotoxicity were made, with an accuracy of 94 per cent. There were 3 per cent false positive reactions and 3 per cent false negative reactions. The phytopharmacologic examination was definitely more reliable than other chemical blood tests hitherto reported (34). These results were presented before the 19th International Congress for Physiology which met in Montreal August 31 to September 4, 1953 (35). Not only was the phytotoxic reaction induced by 1 per cent solutions of the blood serum, but a similar toxicity resulted from saline extracts of skeletal muscle and some other organs when specimens were obtained either from operations or fresh from autopsies. Later studies were made on a series of cerebrospinal-fluid specimens; here again a definite difference in phytotoxicity was found between 2 per cent solutions of the fluid from noncancerous patients and similar solutions from the fluid of patients with malignant tumors somewhere in the body (36).

The foregoing data, involving specimens obtained in all stages of malignancy, indicate that in patients with malignant tumors there is a toxic substance or substances in the blood, spinal fluid, and some of the tissues, which can be

demonstrated by plant-physiologic or phytopharmacologic methods. This test appears to be useful as a diagnostic procedure in addition to other clinical and radiologic methods that are generally employed. Moreover it promises to be useful in evaluation of the distant results of operative, radiologic, and medical procedures in the therapy of tumors.

During the past two years we have been attempting to detect a phytotoxic reaction in the urine of tumor patients. Such experimentation was at first discouraging, because even normal urine is very toxic for seedlings grown in plant physiologic solutions. Nevertheless it was decided first to inquire into the general pharmacologic properties of normal human urine itself. So all of the large number of normal constituents of human urine (37) were tested, separately and in combination with each other, on the growth of *Lupinus albus* seedlings. The results were not as baffling as at first anticipated, because it was found that the main phytotoxic constituent of urine was *urea*. This gave hope of a method for ascertaining or detecting the presence of other phytotoxic constituents, *provided the masking action of urea could be eliminated*. A simple method of elimination was the device of removing the urea by fermentation with the enzyme urease. In this way, when a specimen of urine was tested, first in its original state and then after fermentation, the fermented specimen was much less phytotoxic than the unfermented portion. This was true not only for urine specimens from normal healthy individuals but also for specimens from patients afflicted with various kinds of noncancerous diseases. Similar results were obtained with such urines on testing them, not only on normal plants but also on vernalized plants. This type of experiment was then carried out with urines from cancer patients, and the findings were quite unexpected. When cancerous urines were tested on normal seedlings the findings were usually the same as those with urines of *normal* individuals and of patients with noncancerous diseases. When, however, these urines from cancer patients were tested on *vernalized* seedlings, there was a directly converse finding. The portions of the urine specimens in the unfermented state were *less* phytotoxic for the vernalized seedlings than the urease-treated portions of the same specimens. These findings indicated the activity of a substance or substances (provisionally termed "carcinotoxins") in the urine of patients with malignant disease. In the routine examinations of the blood and urine samples of patients to ascertain the presence of malignancy, the determinations were conducted simultaneously, thus rendering the findings doubly reliable for diagnostic purposes. The full report on these urinary findings will appear in the Journal of Urology.

## CONCLUSIONS

1. The employment of plant physiologic methods in pharmacologic work aids in the study of a number of important clinical conditions and diseases. By data collected on normally reared seedlings of *Lupinus albus* useful diagnostic information has been obtained concerning pemphigus and leprosy, and light has also been shed on the physiology of menotoxin or "menstrual poison."

2. Other studies of a phytopharmacologic character on normally reared plants have revealed the presence of a toxic substance in the blood of psychotic patients, both of the "organic" and "functional" types. Such findings may offer a method of evaluating therapeutic procedures.

3. By employing *vernalized* seedlings of *Lupinus albus*, interesting light has been shed on the pathology of psoriasis and of syphilis.

4. Finally, by studies on such *vernalized* plants, a method has been discovered for detecting the presence of a toxic substance or substances in the blood, cerebrospinal fluid, and the urine of cancer patients. This contributes useful information in regard to diagnosis, prognosis, pathology and evaluation of therapy in malignant disease.

5. The subject of phytopharmacology, even in the present early stages, is an excellent illustration of the successful results which may accrue from a combination of the fundamental sciences of chemistry, physics, zoology and botany with the clinical exercises of medicine, surgery and radiology. The possibilities of such a collaboration for the advancement of medicine are only beginning to be realized. Perhaps it might be well to conclude with a dictum of an ancient sage:

"The work is not upon thee to finish,
Nor art thou free to desist from it."

(R. Tarfon, in "Ethics of the Fathers" II, 21 (about 66 C. E.)

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# RESULTS OF TREATMENT OF PRIMARY ADENO-ACANTHOMA OF THE UTERINE CERVIX, INCLUDING 2 CASES OF ADENO-CARCINOMA ASSOCIATED WITH SQUAMOUS-CELL CARCINOMA "IN SITU"*

ROBERT E. FRICKE, M.D., EDWARD H. SOULE, M.D. AND RICHARD M. CRAIG, M.D.†

*Section of Therapeutic Radiology and Section of Surgical Pathology, Mayo Clinic and Mayo Foundation,‡ Rochester, Minnesota*

Primary carcinomas of the uterine cervix are predominantly squamous-cell epitheliomas, which constitute about 85 to 95 per cent of all such cancers. The remainder are adenocarcinomas, usually arising in the cervical canal, plus a few tumors which show a mixture of squamous-cell and glandular elements, the so-called adeno-acanthomas.

A recent study of 164 cases of adenocarcinoma of the cervix (1) indicates that the gross appearance and symptoms presented by these tumors are identical with those shown by the usual squamous-cell epitheliomas, that apparently adenocarcinomas are somewhat more radioresistant than squamous-cell epitheliomas, and that the stage or extent of the growth is the best prognostic factor regardless of whether irradiation or surgery is the basis of treatment.

In 1940, the data on adeno-acanthoma of the uterus treated at the Mayo Clinic were reviewed (2). The adeno-acanthomas of both cervix and corpus uteri totaled 28, or 1 per cent of the total number of malignant tumors of the cervix and body of the uterus. Symptoms and signs did not differ from those of the more usual cancers. Seventeen of the 28 tumors involved the uterine cervix; radium therapy supplemented by roentgen therapy was the treatment of choice in 11, and surgery in 5. Thirteen patients had been treated at least five years prior to the report; 23 per cent of these had a five-year survival.

Although primary adeno-acanthoma of the uterine cervix is extremely rare, the radiologist may be confronted by such a lesion at any time. It seemed worth while to review the treatments and results in a later group—those seen at the clinic in the years 1945 through 1949. Although around 600 patients with cancer of the cervix were seen in those years, in 13 or about 2 per cent the diagnosis was squamous-cell carcinoma plus adenocarcinoma.

The symptoms and signs of the disease were indistinguishable from those of the usual cancers of the cervix. The diagnosis in all instances was made on the basis of microscopic examination of biopsy material or of surgically removed tissue.

The treatment was mainly radiologic, consisting of radium therapy given by

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† Fellow in Radiology, Mayo Foundation.

‡ The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

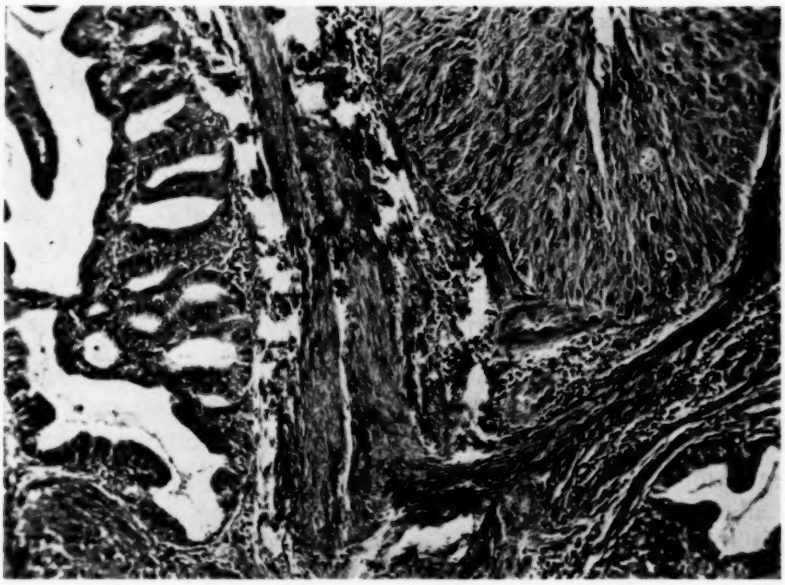

FIG. 1. Adeno-acanthoma of the endocervix, demonstrating an area of grade III squamous-cell carcinoma and grade I adenocarcinoma (hematoxylin and eosin; ×90).

the intensive divided-dose method, supplemented by roentgen therapy. Radium treatments, applied twice weekly to different areas in the cervical canal, corpus uteri and vaginal cavity, were so applied as to give a homogeneous effect throughout the entire birth canal. Treatments took three to four weeks to complete, and the total dosage usually was from 7,000 to 8,000 mg. hours. Four days of high-voltage roentgen therapy followed the radium applications; usually 560 roentgens measured in air were given to each of two frontal and two dorsal pelvic fields. The radium was varied in the case of recurrences or discrete lesions of the vaginal wall by the intravaginal use of flat or curved bakelite plaques containing two or three 50-mg. tubes in parallel arrangement.

When first seen here, 8 of the lesions had been modified by previous treatment elsewhere—by radium and roentgen therapy in 5 instances, by surgery and irradiation in 2, and by surgery alone in 1.

The stage or extent of the disease at our examination was as follows: 5 were classified as stage III, 5 as stage II, 1 as stage I, and 2 as stage 0.

Microscopic examination showed 5 of the lesions to be grade III, and 4 to be grade II adeno-acanthomas. Two lesions were composed of grade I adenocarcinoma with foci of grade III squamous-cell carcinoma admixed with the glandular element (Fig. 1). One of these lesions with mixed grades appeared to be arising from metaplasia of the glandular component, but the tissue removed for biopsy in the second case did not demonstrate the origin of the squamous element, and may have represented an admixture of two separate carcinomas. In 2 other cases the squamous-cell carcinoma appeared as a grade III "in situ" lesion which was confined to the surface of the endocervix involved by the adenocarcinoma.

The treatment given in these 13 cases consisted of radium and roentgen therapy in 7 instances, surgery only in 3, surgery and radium treatment in 2, and no treatment in 1 instance.

The results of treatment in this study were decidedly better than the earlier results reported in 1940 (2). All patients had been treated at least five years previously. Four of the 13 were known to have died, and we were unable to trace 1 patient. The remaining 8 patients were alive and well in 1954, the time of this report.

The patient given no treatment at the clinic had undergone total abdominal hysterectomy elsewhere in 1935 for a cancer showing grade III squamous-cell epithelioma and grade I adenocarcinoma, followed by radium and roentgen therapy. She was examined at the clinic in 1947 and found to have recurrence of the malignant lesion; she preferred further treatment at home. In spite of radium and roentgen therapy at home, she died of carcinoma in 1951, sixteen years after the initial treatment.

Of the 3 patients receiving surgical treatment only, 1 underwent vaginal hysterectomy for a carcinoma-in-situ lesion of squamous-cell epithelioma grade III and adenocarcinoma grade I. Another underwent total abdominal hysterectomy for a mixture of adenocarcinoma grade I and squamous-cell epithelioma grade III in the cervical canal (Fig. 1). The third had a Wertheim operation, with no residual carcinoma found following complete radium and roentgen treatment elsewhere. All 3 patients are living and well for over five years.

Many of these tumors arise in the cervical canal and are infiltrating in character and prone to recurrences. The patient described, who succumbed to cancer sixteen years after the initial treatment elsewhere, died of a recurrence.

FIG. 2. Grade III adeno-acanthoma of the endocervix, demonstrating the mixture of anaplastic squamous and glandular elements (hematoxylin and eosin; ×90).

One patient, aged 37 years, received complete radium and roentgen treatment early in 1945 for a grade III adeno-acanthoma. Seven years later there was a recurrence in the left side of the pelvis, as proved by incision and biopsy. Further radium and roentgen treatment was given in July 1952. She has remained well to date.

Another patient, aged 55 years, underwent a Wertheim operation, including removal of pelvic lymph nodes and a portion of the urinary bladder, in August 1948, for a grade III adeno-acanthoma (Fig. 2). There was a recurrence at the vaginal vault in June 1949. This was apparently successfully treated with radium plaques and roentgen therapy, since the patient appeared well five years later.

## SUMMARY

In the years 1945 through 1949, 13 cases or roughly 2 per cent of the cancers of the uterine cervix treated at the Mayo Clinic were found to be adeno-acanthoma. The histogenesis of these rare cancers is unknown, but the two components may arise from regenerative cells (2). Radiotherapy was employed in most of the patients, although 3 had surgery only, and 1 had no treatment at the clinic. Results of treatment, assessed in 1954, were somewhat better than expected, although 4 patients were known to have died and 1 patient was untraced. The remaining 8 of the original 13 were living and apparently well in 1954—7 for over five years, and 1 for two years after treatment for a recurrence.

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# CLINICAL RESULTS WITH LENTE INSULIN*

MAURICE PROTAS, M.D. AND WILLIAM KURSTIN, M.D.

*Washington, D. C.*†

Progress in the treatment of diabetes began with the discovery of insulin. Insulin permitted better dietetic allowances and better nutrition. Thus the present-day healthier diabetic can live and work right along with the nondiabetic.

Insulin, antibiotic therapy, improved techniques in anesthesia, modern surgical advances, and greater knowledge of electrolyte balances have all been factors in prolonging the life of the diabetic. This prolongation of life, however, has met with a challenge, namely, the increased incidence of the vascular degenerative diseases, the neuropathies and the retinopathies among diabetics. After about ten years of diabetes, the degenerative complications appear.

## TYPES OF INSULIN

The next advance in treatment came with the modifications of insulin. Prolonged action was obtained by adding a protein (protamine, Hagedorn) to insulin and zinc. Later globin was added to insulin and zinc, and finally N.P.H. (neutral protamine, Hagedorn) insulin was produced; the latter two formed the intermediary insulins.

These modifications, however, included a foreign protein. Protamine is a known antagonist to heparin and raises the question as to whether this factor could contribute to thrombotic tendencies and arteriosclerotic involvement of vessels; or whether the foreign protein could account for allergic reactions involving the small capillaries, and thus give rise to the intricate degenerative changes seen in the vascular system, especially in the retinae and glomeruli.

It is possible that the addition of a foreign protein to insulin might in certain patients (usually the thin diabetics with poor glycogen reserves and poor subcutaneous fat depots) fail to disengage itself from the combination with zinc at the usual time of release, but come into play at irregular intervals and thus result in poor control of the blood sugar level. For these reasons, an insulin which is protein-free and capable of prolonged action should be of value. It is recognized, however, that there are numerous humoral agents and other unknown factors which affect blood sugar control and thus interfere with the successful action of exogenous insulin, regardless of the method of preparation.

Lente insulin, the most recent advance in insulin therapy, appears to be a great stride forward in a new direction of insulin modification.

K. Hallas-Møller and associates, of Denmark, presented a series of reports (1–4) of new insulin-zinc modifications, involving elimination of protein and the substitution of acetate buffer for phosphate buffer in the presence of zinc. One

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† Address: 915 19th Street, N.W.

such preparation, a cloudy suspension containing 0.2 mg. of zinc per 100 units of insulin becomes slowly soluble at the pH of blood. In contrast, insulins containing proteins (such as protamine, globin, histones and kyrins) have their prolonged action as a result of the protein combining with insulin to form an insoluble precipitate.

Hallas-Møller substituted acetate for phosphate as a buffer and found that acetate does not have as great an affinity for zinc. With strong phosphate ions, zinc is precipitated out of solution and in such a buffer insulin is available for quick action. The acetate buffer permits a combination of zinc and insulin in such a manner that the final insulin is relatively insoluble at pH 7.2.

The pancreas is rich in zinc. This element is apparently important in influencing the action of insulin. Scott (5) combined zinc with insulin and obtained insulin crystals. This combination, however, did not prolong the action of insulin. Hagedorn combined protamine with insulin and added 2 mg. of zinc per 1000 units of insulin (buffered in phosphate) and did obtain a long-acting insulin.

The discovery of Hallas-Møller and associates (1, 2, 3) led to the development of three new long-acting insulins, prepared in the Novo Laboratories in Copenhagen, and known as Novo-"semi-lente," Novo-"lente," and Novo "ultra-lente." These insulins show retarded action when buffered with acetate instead of phosphate. Their action time depends upon 1) the physical state of the insulin; the larger the crystal, the more prolonged the activity, and 2) the amount of zinc; the greater the concentration of zinc, the more delayed the activity.

The physical state of these insulins is amorphous and crystalline. Semi-lente, the crystalline insulin, is in an amorphous state and has an action time of eighteen hours. Lente insulin acts for about twenty-four hours and is partly in the amorphous state and partly in the crystalline state. Ultra-lente insulin has an action time of thirty hours or more, and is a suspension of insulin crystals measuring 10–20 microns.

An intermediate preparation can be made by combining a crystalline form (which is relatively insoluble and may have an action time of over ninety hours) with an amorphous form (which is more readily absorbed by the body tissues and has an action time of about twelve hours). The pH is adjusted and the zinc content is held at 2 mg. per 1000 units of insulin. The mixture consists of 70 per cent of crystalline form and 30 per cent of amorphous form. This combination results in an insulin similar in action to that of N.P.H. or globin zinc insulin.

## CLINICAL OBSERVATIONS

Our observations on Lente insulin were made during the past year. Forty patients were selected from a group presenting 1) uncontrolled blood sugar levels under current insulin management, 2) thrombotic tendencies, 3) the necessity for multiple doses of insulin combinations, or 4) allergy to present forms of insulin.

The average fasting blood sugar level was 215.6 mg. per 100 cc. (Folin-Wu method) during previous insulin regimens. During treatment with Lente insulin plus quick-acting insulin the average reading was 172.6 mg. This is not a striking

reduction in terms of average readings, but there were diabetics whose fasting blood sugar level remained between 200 mg. and 300+ mg. on a total insulin dosage of 58 units, whereas their average blood sugar level was only 73 mg. on a total dosage of 27 units of Lente insulin.

The total average insulin dose before starting Lente was 37.7 units, and with Lente insulin it was approximately the same—34.4 units. The average amount of crystalline insulin used with the previous regimens was 4.5 units, whereas the average amount of crystalline insulin used with Lente insulin was 7.6 units. (Regular or crystalline insulin was used as a separate injection.)

Lente insulin behaves more like an intermediate insulin and our observations show the onset of action to be between two and three hours after injection, with a peak at eight hours. Its effect increases at a slow rate unless a dose above 50 units is given. The course of action has been described by several of our patients as smooth, but without the usual warning signs of an impending insulin reaction. Reactions have occurred in the early morning hours. Unless a large single dose has been given, the reaction is not intense, and responds rather quickly to orange juice.

"Insulin lumps" have not been observed. In one of our patients, a white female aged 32, with diabetes of twenty years' duration, a stubborn skin eruption developed over the dorsum of each foot, extending upward to just below the knee. This appeared while she was taking protamine zinc insulin and, later, N.P.H. insulin. Within a month after changing to protein-free Lente insulin, the rash had cleared up.

None of our patients has been disturbed with the intense nocturnal hypoglycemia not uncommonly experienced with protamine zinc insulin, but Greenhouse (6) has reported severe and precipitous reactions during the night when large doses of Lente insulin were used to overcome glycosuria.

In our series, there was generally prolonged action with Lente insulin, without

**COMPARATIVE ACTION of INTERMEDIATE and LONG ACTING INSULINS**

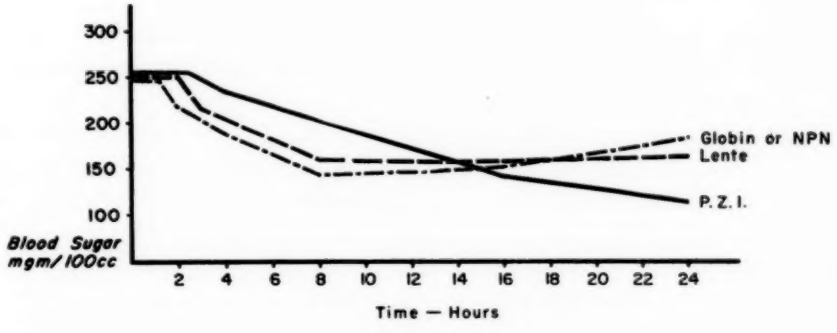


FIGURE 1

the intensity observed after protamine zinc insulin. The fasting blood sugar levels were higher than those observed during treatment with protamine zinc insulin, but lower than those usually seen with globin and N.P.H. insulins. Postprandial glycosuria was quite common.

The change to Lente insulin was made initially on a unit-for-unit basis. Many patients who responded well to Lente insulin, could then reduce the dose by 5 units or more. However, there was complete failure of response to Lente insulin in one of our patients in whom the diabetes had been very difficult to control with either protamine zinc insulin or N.P.H. but fairly well controlled with globin insulin; this necessitated a return to the latter form of therapy. Three more patients in an additional series receiving Lente insulin had to be returned to globin insulin.

Lente insulin has not been prescribed in dosages above 45 units except in 1 patient in whom 58 units was used. In some cases it is still necessary to split the dose of Lente insulin, using $\frac{2}{3}$ of the total dose before breakfast and $\frac{1}{3}$ before supper. In several patients it has been necessary to supplement Lente insulin with regular or crystalline insulin, for postprandial control of glycosuria.

Peck and associates (7) do not advise modifying the Lente preparations by the addition of crystalline insulin, because of the change which occurs in crystal size on alteration of the pH.

Lente insulin was given to a pregnant women in whom the diabetes was complicated with severe bronchial asthma, necessitating the use of Isuprel, Benadryl and other measures for relief of her asthma. The frequency and intensity of her asthmatic attacks were considerably reduced during treatment with this insulin, in contrast to results with the previous insulin.

Holcomb, Page and Stephens (8) reported 1 allergic reaction to Lente insulin, although this was not the experience of Hallas-Møller *et al.* or Oakley (9), who reported complete freedom from allergic reactions. No such reactions were observed in our series.

Diet was regulated in principle according to the usual allowances for N.P.H., globin (10) and protamine zinc insulins, permitting larger evening snacks if hypoglycemic reactions occurred during the night or early morning hours, even though the reactions were usually mild.

## CONCLUSIONS

Lente insulin is a protein-free insulin with a prolonged action of the intermediate type. This new insulin may offer a new approach for the future modification of insulin therapy.

The onset of the action of this insulin is somewhat more delayed than that of globin or N.P.H. insulins. However, its action is more prolonged than that of either of the other insulins, and is not as intense in its nocturnal effect as that of protamine zinc insulin.

It is for the future to determine whether removal of the foreign protein, protamine, will reduce the incidence of thrombotic lesions in the diabetic.

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# THE USE OF RADIOISOTOPES AS TRACER AGENTS IN MEDICAL INVESTIGATION*

FRANKLIN G. EBAUGH, JR., M.D.†

*National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland*

Radioactive and stable isotopes are, and will continue to be, very important agents in medical investigation. Although it is true that some of the studies which are completed through the use of radioisotopes can be carried out by conventional chemical and physical techniques, it is likewise true that many data, now and in the future, will be made possible only through the use of isotopes. The employment of isotopes in medical investigation offers many advantages by virtue of the resultant increased precision, sensitivity, simplicity and efficiency. Isotopes, however, should be considered as only one of many disciplines in medical research. They are certainly no substitute for original ideas and carefully planned and executed experiments. One of the many pitfalls the medical investigator is subject to is over-infatuation with the use of isotopes and all the glamorous and complicated instruments which are involved in their measurement, tending to divert the investigator from following and exploiting the less glamorous but perhaps more important leads pertinent to research. It is obviously impossible to predict the future direction of the uses of radioisotopes as research aids. It will be the purpose of this discussion to point out a few of such uses in some phases of current medical investigation, and no attempt will be made to mention all isotopes of interest. Instead, a few of the basic principles will be discussed, the mastery of which is essential for the employment of these agents in research. The major portion of this discussion will concern the few isotopes with which the author has had some personal experience.

## USE OF ISOTOPES

The following are some of the medical uses of isotopes:

1. For determination of the amount of a substance *in vivo*.
2. For estimation of turnover rates.
3. For tracing metabolic pathways.
4. For making radioautographs.

### *Determination of the amount of a substance in vivo*

Perhaps the simplest illustration of the determination of a quantity of a substance *in vivo* with isotopes is that of the measurement of the blood volume. The following formula illustrates the concepts involved in the measurement of

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† Present address: Dartmouth Medical School, Hanover, N. H.

the volume of the circulating red blood cell mass by the use of sodium chromate$^{51}$-labeled erythrocytes:

$$\text{RBC Mass} = \frac{\text{Total injection CPM}}{\text{CPM/ml. RBC}} = \frac{4.91 \times 10^6}{2.44 \times 10^3} = 2010 \text{ ml.}$$

The erythrocyte (RBC) mass is determined by dividing the total number of isotope injection counts per minute (CPM) by the number of counts per minute per milliliter of circulating red cells in the patient. The quotient will equal the number of milliliters of packed red cells. In this particular example, a figure of 2010 ml. was obtained. This is a rather simple illustration of isotopic dilution.

A more general formula for determining the amount of a substance *in vivo* is illustrated by the following example of the measurement of the miscible pool of iron in a normal female by means of injected $Fe^{59}$.

$$\text{Miscible Pool (M.P.)} = (\text{Amt. injected}) \left( \frac{I_i}{I_0} - 1 \right)$$

where $I_i$ equals the specific activity of the injected isotope and $I_0$ equals the specific activity of the material at "zero time" in the plasma. Specific activity equals the number of nuclear disintegrations per unit time (CPM) per unit weight. Ordinarily, if the number of atoms of the substance injected is small compared with the number of atoms of the substance present in the *in vivo* system, the factor "minus 1" may be safely ignored. As used in this paper, the miscible pool of iron represents the total amount of iron in the patient which rapidly mixes with the injected $Fe^{59}$. In this particular study, a tracer of 6 μg. of iron containing $Fe^{59}$ isotope with a specific activity of $9.04 \times 10^5$ CPM per μg. of iron was injected. The specific activity of the plasma iron at "zero time" calculated by extrapolation to zero time equaled $1.6 \times 10^3$ CPM per μg. of plasma iron. Substituting these values in the foregoing formula, the miscible metabolic pool of iron equaled (6 μg.) $\times \frac{9.04 \times 10^5}{1.6 \times 10^3} = 3.4$ mg. of iron. In order to insure valid results in this type of study, four conditions must be met: 1) the amount of material injected must be small compared to the amount of substance *in vivo* (usually 3 atoms or less per 100 atoms of material *in vivo*) and must not change the rate of metabolism of the substance being studied in the *in vivo* system, 2) the material being studied *in vivo* must be in a "steady state", *i.e.*, the amount removed from the space or compartment being studied must equal the amount entering, 3) there must be no re-entry of the injected isotope into the system being measured, and 4) there must be rapid and complete mixing of the injected material with the same element or chemical compound *in vivo*.

*Exponential or logarithmic functions:* Exponential or logarithmic functions are very important in the study of certain biologic functions. As an example, the physical radioactive decay curve of the isotope $Fe^{59}$ will be discussed.

Figure 1 is a graph of the physical decay of a radioactive isotope, $Fe^{59}$. The vertical axis is in the logarithmic scale and the horizontal in the linear scale. It may be seen that if the activity remaining after any given time is plotted on

the logarithmic scale, the result is a straight line. This straight line has one unique characteristic, *i.e.*, for any given time interval, the percentage drop in the amount of remaining material is constant. For example, at day "zero" there is 100 per cent of the material; at day 1, 98.5 per cent of the material remains—a drop of 1.5 per cent. Now, exactly the same percentage drop occurs at any time during the curve. For example, on the sixtieth day, 41 per cent of the radioactivity remains and on the sixty-first day, 40.4 per cent remains—the difference being 0.6 per cent. The decrement of 0.6 per cent divided by the

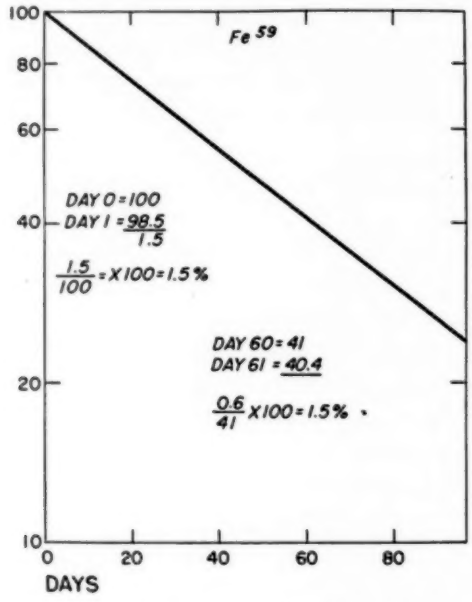


FIG. 1. The radioactive decay of $Fe^{59}$. The ordinate (log scale) represents the percentage of $Fe^{59}$ remaining at time $t$. The time in days is represented by the abscissa (linear plot).

average amount present during this time interval, 41, equals 0.6/41, or 1.5 per cent. This function is described mathematically by the formula $x = e^{-\lambda t}$, where $x$ equals the fraction of the substance remaining at any given time, $t$, the time elapsed from the beginning of the observations; $\lambda$ = the rate constant, and $e$ = the base of natural logarithms, 2.718. In the case of $Fe^{59}$, one-half of the material has disappeared at the end of 46.3 days. Thus, for a period of $t$, 46.3 days, the amount of substance, or $x$, remaining would be 50 per cent. Therefore, 0.5 equals $e^{-\lambda 46.3}$; or, by converting the exponent of $e$ to a positive one, 0.5 equals $1/e^{\lambda 46.3}$; or, expressed in another way, $2 = e^{\lambda 46.3}$. The $\log_e 2$ equals .693; hence, $.693 = \lambda 46.3$; $\lambda = .693/46.3 = .015$ or 1.5 per cent per day. A rate constant ($\lambda$) of .015 or 1.5 per cent was obtained graphically in the preceding paragraph. The formula, therefore, for the decay of $Fe^{59}$ is expressed as $x = e^{-.015t}$ when $t$ is in days; $\lambda$ thus may be considered as equal to the fraction of a given substance which disappears from the system during a given time unit.

*Turnover rates*

*Biologic half-life:* The employment of the formula $x = e^{-\lambda t}$ is of use in determining turnover rates of a variety of substances in the body, provided certain basic criteria are met, which have been outlined in the preceding section. An example which is currently being used in the investigation of iron metabolism is that of the rate of disappearance of intravenously injected $Fe^{59}$ from the plasma.

Figure 2 illustrates three types of $Fe^{59}$ disappearance curves in 1 normal and 2 disease states (1): The middle curve B is that seen in the normal individual. This curve is logarithmic since it is a straight line on semilog paper. The curve for the half-life (disappearance) of the plasma iron (Curve B) represents 1.2 hours, the time at which the plasma $Fe^{59}$ has reached a value of one-half the amount calculated to be present at "zero time." Curve A represents the rate of disappearance of plasma $Fe^{59}$ in a case of bone marrow failure, in which the rate of red cell production, and hence the utilization of plasma iron, was seriously impaired. Here, the rate of decrease of plasma $Fe^{59}$ was far slower, showing a half-life of 3.4 hours. The bottom curve, curve C, illustrates the very rapid disappearance of injected $Fe^{59}$ in patients with iron-deficiency anemia, the half-life being only 0.4 hour.

*Turnover rates:* Once the half-life (disappearance rate) of plasma $Fe^{59}$ is known, as well as the miscible pool, and all the criteria previously outlined are met,

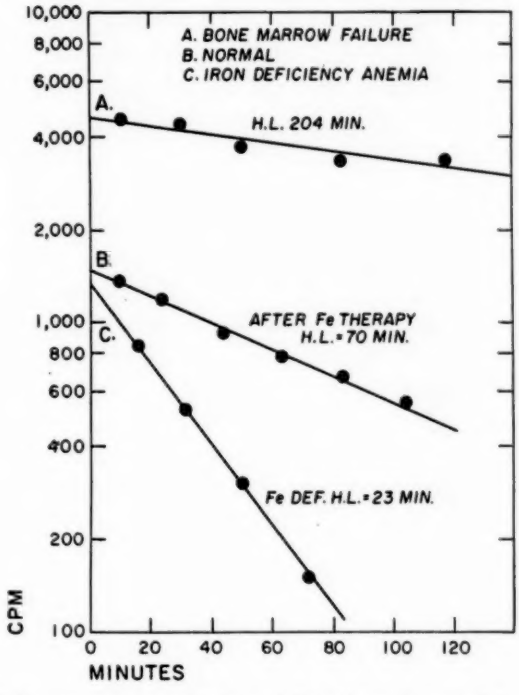


FIG. 2. The rate of disappearance of injected $Fe^{59}$ from human plasma. H.L. = half-life; CPM = counts per minute; Fe DEF. = iron deficiency. (*See* text for discussion.)

the turnover rate of plasma iron can be calculated, as previously shown by Huff (2). The following example illustrates the manner in which the turnover rate of iron in the plasma may be determined. These data were obtained following iron therapy in a female patient who had previously been iron deficient and had become for practical purposes, normal. The level of plasma iron in this patient was 75 μg. per 100 ml., or .75 μg. per ml. As shown in Figure 2, the half-life (rate of disappearance) of the injected $Fe^{58}$ in the plasma, was 1.2 hours. The rate constant or the percentage of the injected material which disappears during any given unit of time is determined by dividing the $\log_e 2$ (or .693) by the half-life of 1.2 hours; in this case it was equal to .58, or 58 per cent per hour. The amount of miscible iron in this case had already been found to be 3.4 mg., in the preceding example. Therefore, the mg. per hour of miscible iron "turned over" equaled the rate constant (.58) times the amount of miscible iron (3.4 mg.); or $.58 \times 3.4 = 2.0$ mg. turned over per hour. Table 1 (1) shows comparative data obtained in a patient with iron-deficiency anemia, before and after iron therapy. Before treatment the serum iron level was 7 μg. per 100 ml.; after treatment, it was 52 μg. per 100 ml. The miscible pool of iron before therapy was 0.54 mg., compared with 2.02 mg. after therapy; thus 0.49 mg. of iron was turned over or removed from the plasma per hour before iron therapy, and 2.08 mg. after iron therapy. It can be calculated that the miscible iron in the patient with iron-deficiency anemia was turned over 22 times in a 24-hour period, in contrast with a turnover of 25 times after treatment with iron. Not all studies of ferrokinetics are as straightforward as the preceding one, since many graphs of disappearance of plasma $Fe^{59}$ do not represent first-order reactions (straight lines on semilogarithmic plots), but are curves and very difficult to analyze. Furthermore, results are often obtained which do not correlate with what is found to be the red blood cell turnover rate as measured by other techniques such as $Cr^{51}$ (1) red blood cell survival.

Unfortunately, many problems in medical investigation are not first-order exponential functions. In fact, the vast majority of observations are not simple first-order reactions, but may exhibit several independent processes occurring at once. Furthermore, frequently a steady state does not exist and there may be interference with the naturally occurring metabolic processes by the injected material. The isotope may be reutilized, and complete mixing of the isotope

TABLE 1

*Effect of iron therapy on ferrokinetics in the human*

| | Before Fe | After Fe |
|---|---|---|
| Plasma Fe (μg./100 ml.) | 7 | 52 |
| Miscible pool (mg.) | 0.54 | 2.02 |
| Turnover (mg./hr.) | 0.49 | 2.08 |
| No. of times the miscible pool was turned over in 24 hrs. | 22 | 25 |

with the naturally occurring substance may never be complete, or may occur so slowly as to vitiate the study.

Sometimes, useful approximations of turnover rates can be made when ideal conditions do not exist, as in the majority of studies of the *in vivo* survival of erythrocytes, one example of which is shown in Figure 3. In this case, erythrocytes from a patient with cirrhosis of the liver were labeled *in vitro* (3, 4) with $Na_2Cr^{51}O_4$ and injected into the same patient. The percentage of the original amount of radioactivity (corrected for elution of $Cr^{51}$ from the circulating erythrocytes) remaining in the blood at various intervals after the injection was plotted against time (Fig. 3). A semilogarithmic plot of this curve does not follow a straight line. Since this is a complex curve, being neither linear nor

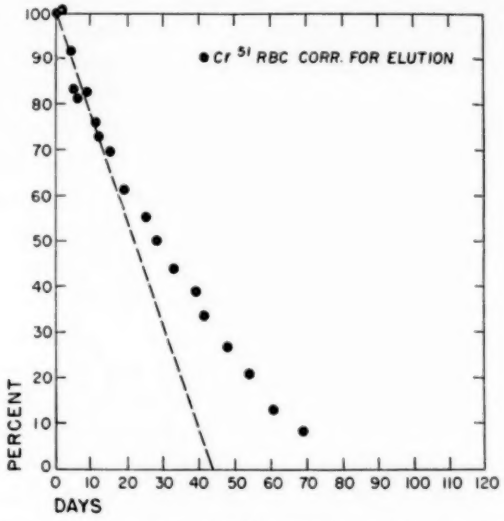


FIG. 3. The survival of autotransfused $Cr^{51}$-labeled erythrocytes in a patient with Laennec's cirrhosis of the liver. (*See* text for discussion.)

logarithmic, exact analysis is difficult. An approximation of the mean red blood cell life-span can be made by extending a tangent to the initial slope of the curve to the time axis (5). The term red blood cell mean life-span is defined as the average period of time the red cells live in the circulation, and this term should not be confused with the *maximum* life-span of a circulating red cell which, in the case of the data presented in Figure 3, is considerably in excess of the mean life-span of forty days. Although only approximations, such studies are of value in estimating the turnover rates of erythrocytes in various types of anemias. In this instance the mean life-span of the red cell in this patient with cirrhosis was about forty days, compared to a normal value of ninety to one hundred and twenty days (6). These data, expressed in another way, indicate that about 2.5 per cent of the patient's red cells were destroyed each day, compared to a normal figure of .8 to 1 per cent. If during this study the patient's hematocrit remained at the same level and no significant amount of blood loss

occurred, then it could be concluded that the rate of red blood cell production equaled the rate of destruction, and the turnover rate of the red cells per day could be estimated by multiplying the total red cell mass by .025.

*Metabolic pathways*

The employment of isotopes as tracers for metabolic pathways can be only briefly mentioned. An example is shown in Figure 4, in which Dr. Marjorie Stetten (7) used the stable isotope, $N^{15}$, to determine by which pathway the formation of proline from ornithine took place. The two possible intermediate compounds were $\alpha$-keto-$\delta$-aminovaleric acid and glutamic semialdehyde. The problem was solved by preparing two differently labeled ornithine molecules—one with the alpha nitrogen (the $NH_2$ group to the reader's right in ornithine, Fig. 4), the other with the delta nitrogen (the $NH_2$ group to the reader's left in the ornithine molecule), enriched with $N^{15}$. The two labeled ornithine molecules were then fed to animals and the content of $N^{15}$ was determined in the proline isolated from the proteins of the animal nitrogen. It was found that there was more $N^{15}$ present in proline when alpha-$N^{15}$ labeled ornithine was fed than when delta-$N^{15}$ ornithine was fed. This investigation clarified the pathway of the transformation of amino groups from ornithine to proline and corrected the misconception that the delta-amino group of ornithine was the chief source of nitrogen for proline derived from ornithine.

*Radioautography*

Figure 5 shows data obtained by Drs. Robbins and Rall (8), in their studies on serum iodine. The upper portion of Figure 5 is a paper electrophoretic pattern of serum from a patient who had thyroid carcinoma and who received a large dose of $I^{131}$, resulting in the release of labeled thyroglobulin. In performing

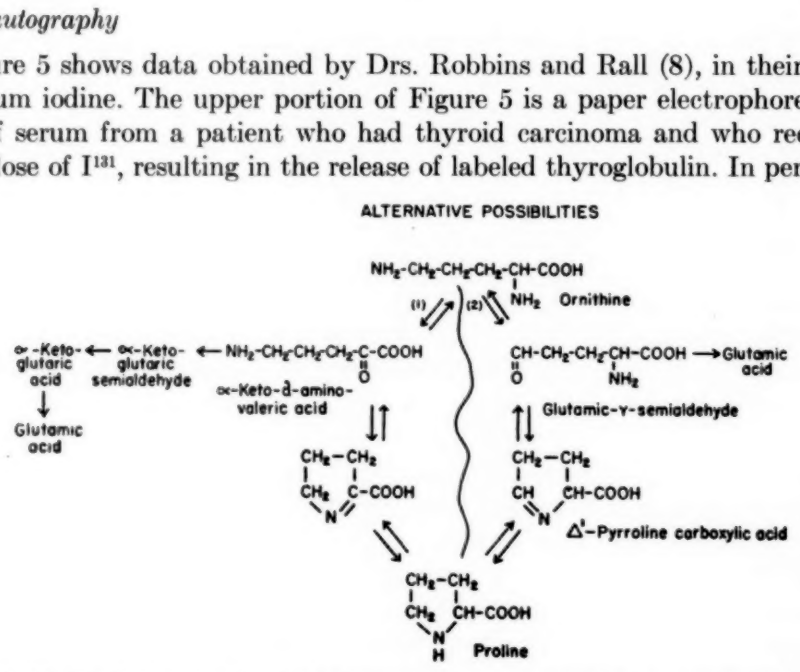


FIG. 4. Alternative routes for the interconversions of ornithine and proline. (Reprinted with permission, from the article (7) by Dr. Marjorie Stetten in *A Symposium on Amino Acid Metabolism* edited by McElroy and Glass. Baltimore, the Johns Hopkins Press, 1955.)

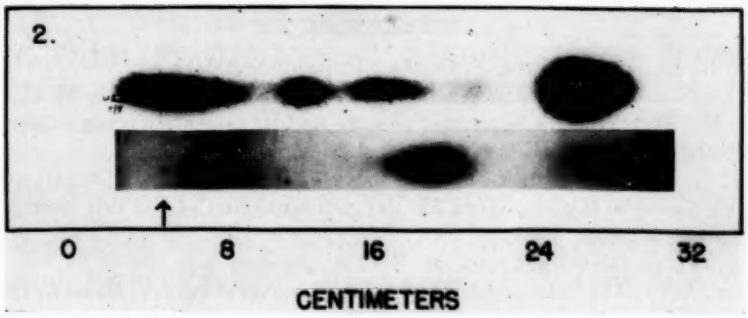


FIG. 5. The distribution of protein-bound $I^{131}$ in the serum (8). The upper portion of the figure is the paper electrophoretic pattern of serum. The albumin is the dark spot to the right; it has migrated farthest from the origin at the left towards the positive pole to the right of the figure. In the bottom strip is a radioautograph of the upper paper electrophoretic strips. The darkest spot in the bottom strip represents $I^{131}$-labeled thyroglobulin and shows that thyroglobulin occurs in the alpha globulins of the serum. There is a small amount of activity at the far-right end of the strip, representing some $I^{131}$ present in the serum albumin. The darkness at the left of the bottom strip is an artifact and not caused by $I^{131}$. (Reprinted with permission, from the article (8) by J. Robbins and J. E. Rall in the Proceedings of the Society for Experimental Biology and Medicine, vol. 81, p. 530, 1952.)

paper electrophoresis, a small amount of serum is placed in the center of a strip of the filter paper, and an electric current (D.C.) is then applied. The upper part of Figure 5 represents only half of the filter paper strip, so the point of origin is on the left-hand side of the picture. In this experiment, the serum albumin migrated the greatest distance towards the positive pole (right-hand side, Fig. 5) followed by alpha-1, alpha-2, beta, and gamma globulins. The radioautograph (bottom half of Fig. 5) was made by placing the paper strip onto an x-ray film, allowing an adequate time of exposure, and developing the film. The dark spot immediately below the alpha globulins was caused by the $I^{131}$ present in the thyroglobulin. Thus, the location of thyroglobulin in the serum was found to be in the alpha globulin. Radioautographs have a wide application in electrophoresis, chromatography, and histologic techniques for the specific localization of chemical elements and compounds labeled with radioactive isotopes in various systems.

## CONCLUSION

The principles and a few examples of the use of radioactive and stable isotopes in medical investigation have been presented. The continued use of these isotopes as tracers will undoubtedly lead to the accumulation of much new information, particularly in the field of intermediary metabolism, and will ultimately result in practical advances in diagnosis and therapy.

*Acknowledgments*

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# THE THERAPEUTIC USE OF RADIOISOTOPES FROM A PRACTICAL LEVEL*

E. R. KING, CAPT., MC, USN

*Radioisotope Laboratory, U. S. Naval Hospital, Bethesda, Maryland*

Radioisotopes have been used in therapy for various conditions, both benign and malignant, for approximately fifteen years. In the early days of their use, great publicity was given to this new method of treatment and as with most new drugs the enthusiasm of some workers was not completely justified. Then there was a period of more or less disinterest by many of the medical profession and during this period the workers with radioisotopes were able to complete a more logical evaluation of the application of these substances.

In discussing the modern practical applications of isotopes one should discuss first the rationale, and in order to discuss the rationale, one must consider isotopes as a source of radiation which can be placed inside the body or more preferably inside a tumor mass. The radiation emitted comes from the interior and irradiates a relatively small amount of healthy tissue around the periphery of the tumor. In x-ray therapy the situation is reversed and the useful x-ray beam comes from the outside, irradiating a great mass of healthy tissue prior to reaching the tumor and also another mass of healthy tissue on the other side of the tumor. Consequently with radioisotopes it is possible to a certain degree to center the radiation in the tissue or organ being treated, thus sparing healthy tissue. If this were true in all cases we would indeed have an ideal set-up for radiation therapy of malignant tumors or of those benign conditions in which radiation therapy is indicated. Unfortunately, the situations in which this ideal condition occurs are all too few.

In addition to describing the ideal situation for using isotopes, one might discuss what would be an ideal isotope for therapeutic procedures: 1) Such an isotope should not be toxic to the human body. It is known that some elements are extremely toxic and in administering isotopes of these elements to human beings, one might poison the patient with the stable element of the radioisotope used. 2) The isotope should be of a biochemical and chemical nature such that a compound could be prepared from it that would be biologically acceptable to the individual. In other words, if it were given intravenously, the isotope or compound should be prepared as a solution with a neutral pH, and should not compete with, or remove from the blood the necessary constituents that are circulating in the blood stream. If the radioisotope be given orally, it or the compound in which it is to be administered should be soluble and be absorbable by the gastro-intestinal tract. Another type of compound may be prepared as a colloid and because of the size of its molecules will remain within the body cavity when it is placed there. 3) An ideal therapeutic isotope should have a

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half-life lasting from a few days to a few weeks. If the half-life is less than a few days the compound may not be of practical use, in that much of it will have undergone radioactive decay before it can be transported to the hospital or clinic that will use it. If the half-life is over a month, the isotope may present a health hazard to the people handling it, as well as an added health hazard to the patient to which it is to be given. 4) The ideal isotope used in therapy should have a strong beta emission, and fairly weak gamma emissions. If it is to be located in a particular tissue in order to irradiate that tissue, it should spare the surrounding healthy areas. If strong gamma emissions arise from the isotope, their penetrating power will be very great and the entire body will be irradiated. 5) Another, and equally important requirement for this type of isotope is that it possess biologic properties that allow localization in the particular organ or tissue to be treated. The only two known isotopes available which have this latter quality are phosphorus$^{32}$ and iodine$^{131}$.

Needless to say, it is difficult to find an isotope for use in therapy which has all of these qualifications. In therapy, from a practical standpoint, there are three isotopes which are used routinely and have proved their worth. These are phosphorus$^{32}$, gold$^{198}$, and iodine$^{131}$. They are used internally or placed internally in the body spaces. Other isotopes are utilized as external radiation sources, such as cobalt$^{60}$, and cesium$^{137}$. In addition, gold$^{198}$ has been used in the metallic gold form as an interstitial source of radiation and a substitute for radon. Cobalt$^{60}$ has also been used as a metallic source in the form of needles or seeds, as a substitute for radium. However, external radiation sources and interstitial sources of irradiation will not be discussed in this paper as it is felt that they are a form of therapy to be employed only by radiologists.

## PHOSPHORUS$^{32}$ ($P^{32}$)

Phosphorus$^{32}$ has a half-life of fourteen days and emits only a strong beta particle. It most nearly fulfils the qualifications of the ideal isotope. Radiophosphorus was one of the earliest used isotopes in medical therapy. Since it is known that phosphorus is an important factor in the metabolism of bone, it was postulated that radiophosphorus might be deposited in bone and be used as a source of radiation, not only to the entire skeleton but, more important, to the bone marrow. Thus the hemopoietic system could be irradiated by oral or intravenously administered $P^{32}$. The early trials were justified and $P^{32}$ is now a treatment of choice for polycythemia vera, and in many instances for chronic myelogenous and chronic lymphatic leukemia. Originally it was administered intravenously because the radiophosphorus was cyclotron-prepared and thus valuable and difficult to obtain. Now that phosphorus is obtained from the nuclear reactor it is practically always administered in water by the oral route. Although a certain amount, probably 20–25 per cent is lost through the feces, the substance is so inexpensive that we need not worry about this loss. In leukemia, the doses are given in varying amounts. In general, the dosage is estimated on the basis of body weight, the average dose being approximately 1 millicurie per 10 kilograms of body weight. $P^{32}$ was often given in one dose and

the patient was followed at intervals until the white blood count dropped to the desired level. At the present time it is felt that better results may be obtained by giving fractions of the dose at intervals and extending the total period of administration. Consequently, an initial dose of 2 or 3 millicuries is given, the patient is followed for two to four weeks and then another dose of 2 to 3 millicuries is given. This is repeated until the white blood count has dropped to the desired level and is maintained there. Another method is to give 1 millicurie per week, following the patient with repeated blood counts and clinical examination until the white blood count drops to 30,000 to 40,000. This method is probably more effective with the myelogenous type of leukemia than with the lymphatic.

X-ray treatment of the splenomegaly which accompanies leukemia, is a matter of choice. It is the opinion of this observer that protracted x-ray treatment of the spleen increases any existing hazards of overtreatment. Moreover, in myelogenous leukemia, splenomegaly can be controlled by radiophosphorus. However, in lymphatic leukemia this does not always hold true and the splenomegaly may persist. Chronic lymphatic leukemia does not seem to respond as well to the use of radiophosphorus as does the chronic myelogenous type.

In the treatment of polycythemia vera, $P^{32}$ appears to be the therapy of choice. The dose given is of the order of 2 to 3 millicuries and the patient is observed for eight to twelve weeks. At the end of this interval another dose of 2 to 3 millicuries may be administered if there has been no remission. A longer observation period is required in polycythemia vera than in leukemia because of the longer life-span of the red blood cell compared with that of the granulocyte or lymphocyte. In treating polycythemia vera, the 2 to 3 millicurie dose may have to be repeated three or four times at two-month to three-month intervals until the red blood cell count is maintained at a normal level. Once the remission has been achieved, it may persist for a period of one to four years before another course of treatment is necessary. The question is always asked as to whether it is advisable to perform venesections in the younger patients with polycythemia vera prior to starting the $P^{32}$ therapy. In the younger age groups venesection is probably the treatment of choice, at least as long as it can control the disease. The incidence of leukemia in cases of polycythemia vera treated with $P^{32}$ is no higher than the incidence in untreated cases of polycythemia vera.

For intracavitary therapy for malignant effusions, $P^{32}$ may be administered in the form of chromic phosphate in which the phosphate is in the form of radiophosphorus, as a colloidal solution.

## GOLD$^{198}$ ($Au^{198}$)

For several years, the use of a radioactive colloidal solution of $Au^{198}$ has been employed for treatment of effusions complicating certain types of cancer. The most common of these are pleural effusion caused by carcinomatosis from breast carcinoma, lung carcinoma, or malignant lymphoma, and the ascites caused by carcinomatosis from ovarian carcinoma, carcinoma of the bowel, or

malignant lymphoma. This form of therapy has been a palliative measure; in approximately 60 per cent of the cases treated, the fluid production can be adequately or partially controlled. In this procedure, radioactive gold, which has a half-life of 2.7 days and emits moderately energetic beta and gamma rays, is injected directly into the pleural or peritoneal space following a thoracentesis or paracentesis. If $Au^{198}$ is used, the customary dose is in the vicinity of 100 millicuries. The patient is then rotated in the bed on all sides, then head down and head up, in order to distribute the colloidal solution within the body cavity in an attempt to expose all of the pleural or peritoneal surface to the radioactivity. Following this treatment it may be necessary to perform one or two additional paracenteses on these patients, as the fluid continues to form for a short time.

The present attitude at our clinic is to treat these patients as early as possible, once a positive diagnosis of a malignant effusion has been made. If the patient is also suffering from generalized carcinomatosis, he should be considered incurable and any possible steps should be taken to afford palliation. Some clinics prefer to wait until after several taps, in order to demonstrate a free and rapid formation of fluid prior to treatment. Our experience has been that the best results are obtained when the patient is treated early; there is no justification for subjecting such hopeless cases to more than one or two fluid taps. Also there is no reason why these patients should be deprived of the vital blood protein lost in these fluids.

Another radioactive colloidal preparation that is being used at the present time is radioactive chromic phosphate, in which the source of radioactivity is radiophosphorus. This would seem to offer a better source of radiation for a longer period of time than radioactive gold, as the beta particles liberated by the $P^{32}$ are much more effective in treating cancer of the serosal membrane than are those of $Au^{198}$. In addition, since $Au^{198}$ emits strong gamma rays, the whole body of the patient receives an appreciable dose of irradiation when it is used. However, two things can be said in favor of the use of gold as compared with the use of chromic phosphate. First, in delivering any type of radiation therapy the time during which the radiation is given is of most importance. It has been demonstrated that in treating any cancer, a radiation dose delivered over a short period of time is more effective than the same dose delivered over a longer period of time. For instance, the cancericidal dose to squamous-cell carcinoma, for radiation delivered in one day, is 2750 roentgens; but the cancericidal dose for the same carcinoma, if the irradiation is delivered in a week, is 4000 roentgens; and if this same cancer is treated over a period of one month, 6000 roentgens will have to be delivered for a cancericidal dose. A glance at the half-lives of $Au^{198}$ (2.7 days) and $P^{32}$ (14 days) reveals that the time required for the total dose of irradiation to be delivered, from the beginning of therapy until complete decay has almost occurred, will be about ten days for $Au^{198}$ and about ten weeks for $P^{32}$. Thus the dose which will be delivered by the radiogold in a two-week period will be more biologically effective than a similar dose delivered by radiophosphorus in the required ten-week period. In many of the patients

fluid continues to form after their first treatment. This means that in a week or ten days it is often necessary to perform another thoracentesis and thus withdraw some of the radioactive substance which had been injected, but which has not undergone complete radioactive decay. If colloidal $Au^{198}$ has been used, most of the radiation will already have been delivered; but if chromic phosphate has been administered, it will not have undergone a complete half-life phase, and thus it is possible that much of the radioactive material may be removed when the paracentesis is performed. This is not completely true, however, as it has been demonstrated that when chromic phosphate is used, some of the colloidal radioactive material clings to the serosal linings and is not withdrawn during subsequent taps. Despite these observations which indicate that $Au^{198}$ would be more effective, the fact remains that this isotope does emit strong gamma rays and therefore irradiates much of the healthy tissue of the body of the patient. Also, because of the strong gamma rays and the large doses required in treating the patients, extreme caution must be used by the handlers and technicians, and it is thus more difficult to administer than is radioactive chromic phosphate. $Au^{198}$ is practically always given by a remote control method or by heavily leaded syringes. Chromic phosphate with $P^{32}$ can be administered by an ordinary glass syringe with little or no shielding of the radioactive material. Most clinics which do not have elaborate shielding facilities or elaborate apparatus for remote-control administration of an isotope will find that radioactive chromic phosphate is much simpler and cheaper to handle than radioactive colloidal gold.

## IODINE$^{131}$ ($I^{131}$)

$I^{131}$ has an eight-day half-life and emits both beta and gamma rays. Its primary therapeutic use is in the treatment of diffuse toxic goiter; in which condition, the results have been good. We shall discuss briefly the types of hyperthyroidism in which $I^{131}$ is indicated.

In most clinics, because of the possibility of genetic effects and the very remote possibility of delayed carcinogenic effects in the thyroid, hyperthyroidism is not treated by $I^{131}$ in young patients and those in the early child-bearing period. In our clinic, unless the patient cannot be adequately prepared for surgery or is a surgical risk because of conditions other than hyperthyroidism, we do not treat any patient under the age of 35 years, nor any woman over 35 if there is any probability she will bear children after that age.

Toxic nodular goiter and nontoxic goiter are not treated with $I^{131}$ as a routine procedure. There is no indication for treating nontoxic nodular goiter with iodine. In toxic nodular goiter there is a possibility that if the nodule is solitary, it may be malignant. Toxic nodules tend to recur after therapy with radioiodine and repeated courses are usually needed to treat these recurrences; therefore clinicians usually prefer not to treat toxic nodular goiter with radioisotopes. However, if the patient is a poor surgical risk, a trial of $I^{131}$ is justified.

The dosage is difficult to determine. It should depend upon the weight of the gland to be treated, but it is very difficult to estimate this weight despite

the use of scanning mechanisms and careful palpation by experienced surgeons. In our experience, it is preferable to give a small dose, realizing the possibility that in two or three months it will have to be repeated. A dose of 5 millicuries is usually employed, and after a three-month interval the patient is re-examined, and thyroid function re-assessed. If a remission has not occurred, another dose of 3 to 5 millicuries is administered, depending upon the condition of the patient. Our clinic has found this method very effective, and less than 10 per cent of the cases have required more than two doses of radioiodine. In some cases the gland is greatly enlarged by the diffuse toxic goiter and a dose of 7 to 10 millicuries is employed initially. Here again, it may be necessary to administer a smaller dose three months later.

In discussing the use of $I^{131}$ versus surgery in the treatment of diffuse toxic goiters most clinics and most thyroid surgeons accept radioiodine as the treatment of choice. This is particularly true in that disturbing age group which includes climacteric and post-climacteric patients, and also patients in whom there is a higher incidence of cardiovascular diseases. With $I^{131}$ therapy there is no scar, there is no damage to the laryngeal nerves, and there is no danger of losing the parathyroid glands.

Another use of radioiodine is the treatment of congestive heart failure, angina pectoris and other types of cardiovascular disease. The rationale of this procedure is that, by partially destroying the thyroid gland the metabolic rate of the body is lowered, and thus the patient and the patient's heart are forced to a lower activity level. This type of treatment has been widely used, particularly in the recalcitrant patient who cannot be forced to lessen his activity and the work load being placed on his heart. In heart disease the dose is somewhat higher than in hyperthyroidism, as the object is to reduce the patient to a myxedematous or nearly myxedematous state. When the thyroid is of average size, we usually administer a first dose of radioiodine in the order of 10 millicuries. After a two-month period of observation, if beneficial results are not obtained, we give another 5 or 10 millicuries, depending upon the condition of the patient. In the older patient it is usually necessary to increase the initial dose and perhaps to give 10 to 15 millicuries as a second dose.

The treatment of metastatic thyroid cancer with radioiodine has been successful in a limited number of cases. If the metastasis is in the neck or chest and can be removed surgically, every attempt should be made to use surgery prior to the use of radioiodine. Radioiodine should be reserved for distant and generalized metastatic disease from carcinoma of the thyroid, in which it is impossible to perform surgical resection of the diseased portion of the body. Even in such cases, the use of $I^{131}$ is limited to the type of cancer which produces colloid. Prior to the treatment of these metastases it is necessary to remove the normally functioning thyroid tissue from the neck, for this will act in competition with the metastasis and will remove most of the radioiodine from the blood stream, allowing little or none to be deposited in the distant lesions. Furthermore, it has been found after removal of the normally functioning thyroid from the neck, that if the primary malignant growth was of the type

producing colloid, the metastatic area may undergo metaplasia with follicular formation and production of colloid.

After the normally functioning thyroid has been removed, the patient should be studied with tracer doses of $I^{131}$, and it should be ascertained whether or not the metastasis is removing the radioiodine from the blood stream. If such removal is observed, one should then treat the metastases vigorously. An initial dose in the order of 100 millicuries is usually administered by the oral route. Following this, the patient is re-examined after a period of approximately three months, in order to determine if the dose has been effective. If it has been even partially effective, our present policy is to administer another 100 millicuries of $I^{131}$. Doses can be administered at three-month to six-month intervals, as long as the metastases appear to be decreasing in size, and if the patient's hematopoietic system can stand this large amount of radiation. Once the white blood cell count falls below 3000, there should a careful re-evaluation of the case. The progress of treatment should be followed, not only by tracer studies repeated at three-month intervals, but also by x-ray and clinical examinations.

## CONCLUSIONS

1. In selecting a radioisotope for clinical therapeutic administration, the following factors should be considered: toxicity of the parent element, biochemical and chemical nature of the parent element, half-life of the radioisotope, energy and type of emissions of the radioisotope, and the probability of biologic localization of the isotope in the desired organ or tissue.

2. The only radioisotopes which are routinely used in clinical therapeutic procedures at present are phosphorus$^{32}$, gold$^{198}$, and iodine$^{131}$.

3. Despite this restriction, these isotopes furnish acceptable treatment for the following conditions: polycythemia vera, chronic myelogenous leukemia, chronic lymphatic leukemia, effusions complicating spread of various malignant conditions to the serosal membranes of the body, hyperthyroidism, metastatic thyroid cancer, and some forms of chronic heart disease.

# THE PRACTICAL USES OF RADIOACTIVE ISOTOPES FOR DIAGNOSIS*

ROBERT J. FINK, M.D.

*Hunter Memorial Laboratory, Washington, D. C.*

By practical uses of radioisotopes I mean those which are of immediate clinical value as opposed to those on a research level that will later become widely useful.

## TESTS OF THYROID FUNCTION

The first and most popular application is for determination of thyroid function by means of tracer doses of radioiodine ($I^{131}$). A measured dose (50 $\mu$c.) of $I^{131}$ is given to the patient. It is taken up by the blood and carried to the thyroid gland, where in a twenty-four hour period about 10 to 45 per cent is retained. The amount can be determined by placing a scintillation counter at a measured distance directly in front of the gland.

During the 24-hour period some of this iodine is bound to protein in the gland, and excreted into the blood. This provides another means of measuring function. A sample of blood is withdrawn from the patient and the level of $I^{131}$ in whole serum is measured. Using trichloracetic acid, a protein extract is then obtained, which contains the protein-bound $I^{131}$; this too is counted for radioactivity. The ratio of the two radioactivities is then determined (normal, 13 to 45 per cent) (1, 2).

There is a another test that is a more indirect means of measurement. The inorganic $I^{131}$ not handled by the thyroid gland is being constantly secreted by the salivary glands, reabsorbed, and re-secreted (3). If a sample of saliva be taken, its radioactivity can be compared to that in the protein-bound fraction of the serum: the ratio is normally about 160 to 235 (4).

Measurements can also be made of the amount of $I^{131}$ excreted in the urine in the first twenty-four hours. This normally constitutes 40 to 65 per cent of the dose (5).

Of the entire dose of $I^{131}$, one may be unable to account for as much as 10 per cent. The fate of this fraction is theoretical.

I have designed a tabular graph which is called the "Thyroid Profile." When the diagnosis is difficult, or a single determination is borderline, several tests may be performed, as in the case of liver disease. Interpretative remarks are usually necessary, since single values may be out of line. It will be noted that there is a broad band on each side of normal (euthyroidism), representing areas where results are considered questionable.

## MEASUREMENT OF FLUID SPACES

Plasma volume may be measured by means of human albumin which has been tagged with $I^{131}$ (6). Red cell mass may be determined by tagging the

* Read before the Fifty-Sixth Annual Meeting of the American Therapeutic Society, Atlantic City, N. J., June 2–5, 1955.

*Thyroid Profile*

| | Thyroidal Uptake $I^{131}$ (%) | Serum $PBI^{131}$ (%) | Ratio Salivary $I^{131}$ to $PBI^{131}$ | Serum Chem. PBI (μg./100 ml.) | BMR (%) | Serum Chol. (mg./100 ml.) |
|---|---|---|---|---|---|---|
| Hyperthyroidism | 50 | 50 | 40 | 8.0 | +15 | |
| | | | | | | |
| | 40 | 40 | 160 | 7.8 | +10 | 150<br>175 |
| Euthyroidism | 15 | 15 | 235 | 4.0 | −10 | 250 |
| | | | | | | |
| Hypothyroidism | 10 | 10 | 338 | 2.5 | −15 | |

FIGURE 1

red cells with $Cr^{51}$ (7). The extracellular fluid space may be measured with $S^{35}$. Total body water may be measured with water labeled with $H^3$.

The radioactive albumin generally is retained in the plasma; the tagged red cells remain with the red cell mass; the $S^{35}$ generally is diluted in the extracellular compartment; but the labeled water goes everywhere in the body.

The procedure for plasma volume determination is illustrative of the other determinations. A measured amount of human albumin tagged with $I^{151}$ is injected into a standard of known volume, and a duplicate sample is injected into the patient's blood, which has an unknown volume. After ten or fifteen minutes is allowed for mixing, equal-sized samples of the standard and of the patient's plasma are obtained and their activities determined. The following formulae are then applied:

1. $$\frac{\text{Counts per min. of standard} \times \text{vol. of standard}}{\text{Counts per min. of plasma}} = \text{Plasma Volume}$$

2. $$\frac{\text{Plasma volume}}{1 - \text{corrected hematocrit}} = \text{Total Blood Volume}$$

3. $$\text{Total Blood Volume} - \text{Plasma Volume} = \text{Red Cell Mass}$$

The red cell mass may be measured more directly by drawing blood from the patient, incubating it for forty-five minutes with $Cr^{51}$, and re-injecting it into the patient. From this point on, the general principles are similar to those used for the plasma determination, except that the result is red cell mass.

The estimation of extracellular fluid space with $S^{35}$ and total body water with $H^3$ are more difficult procedures, because the soft beta rays emitted by these isotopes are not so easily measured as the gamma rays of $I^{131}$ and $Cr^{51}$.

## CLINICAL APPLICATIONS

These values are practical in determining fluid balance in major surgical or burn cases, in the regulation of fluids in patients with cardiac decompensation or kidney failure, and in the diagnosis of hematologic diseases such as polycythemia.

In cardiovascular disease the isotopes may be used to determine circulation time (8). Radioactive iodinated albumin is rapidly injected into a vein and the time is noted. It may be detected and timed when it reaches the heart by means of a scintillation counter over the precordium. It may be detected and timed again by another instrument when it reaches an extremity. Thus the circulation time is obtained.

Radioactive iodinated albumin may also be used to determine the patency of the circulation to an extremity (8). After injection, counts are obtained over the extremity concerned and compared to counts obtained over a similar area on the normal opposite extremity. If lesser activity is detected over the extremity in question, it is assumed that the decrease is due to decreased circulation.

One of the earliest uses of isotopes clinically was in localization of tumors. Metastases from carcinoma of the thyroid were detected because they picked up radioactive iodine. This may seem to be a simple, straightforward test but there are several inconsistencies. First, metastases usually do not pick up detectable amounts of a tracer dose if any thyroid tissue remains in the normal site to compete for it. Secondly, highly anaplastic, non-functional tumors will not pick up iodine. Thyrotropic hormone stimulation or propylthiouracil treatment is sometimes of help in increasing metastatic $I^{131}$ uptake in such cases.

Well vascularized brain tumors (9) and metastatic malignancies (10) are sometimes detectable by this method, since they retain larger amounts of radioactive iodinated albumin than does normal tissue.

Tumors in the eye may be differentiated from hemorrhage behind the retina, because they retain more $P^{32}$ (11). Other tumors also retain $P^{32}$, but unless they are close to the skin surface the poorly penetrating beta rays cannot be detected. Therein lies the value of $I^{131}$-tagged albumin, because its gamma rays easily penetrate the bony covering of the brain. Recently a more promising isotope has been found for localization of brain tumors, namely, positron-emitting arsenic$^{74}$ (12). The arsenic is retained by the brain tumor and emits positrons. Positrons undergo immediate annihilation along with the first electron with which they collide. Two gamma rays of equal strength are produced simultaneously at right angles to the collision. These are detected by two scintillation counters which are so connected electronically as to detect only those impulses received simultaneously. This results in a high degree of resolution, since all other impulses are ignored.

There are some other tests which might be considered practical in larger laboratories. The metabolism of iron in hemosiderosis and the various anemias usually requires more equipment than the average laboratory will find practical, except on a research basis.

The biologic half-life of (radioactive) albumin may become an important determination in liver disease (13). It has been found to be prolonged in cirrhosis.

The digestion of (radioactive) albumin by the pancreas may later become a practical diagnostic tool as a measure of pancreatic function (14).

The survival time of red cells in the various hemolytic disorders can be determined by tagging the cells with $Cr^{51}$ (15). Measurement of the survival time is carried out by taking repeated samples of blood until only half the activity remains, using the formula:

$$4. \quad \text{Per cent survival} = \frac{(\text{cpm of whole blood at "x" time})(e^{\lambda t})}{\text{cpm of whole blood at 24 hours}} \times 100\%$$

A curve is drawn and the life span of the red cells is determined.

## SUMMARY

Radioactive isotopes can be used to measure thyroid function, determine fluid spaces, measure circulation time, test the patency of circulation, localize tumors and metastases, and to furnish data in other metabolic studies. Many of the results are of immediate practical clinical value, and many more tests now being conducted on a research level will become clinically adaptable in the future.

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# INTRA-ARTERIAL VASODILATOR AGENTS IN THE TREATMENT OF ADVANCED OCCLUSIVE ARTERIAL DISEASE OF THE EXTREMITIES

## A Preliminary Report of a New Vasodilator Drug, 27 MI*†

O. ALAN ROSE, M.D. and ALFRED EBEL, M.D.

*The Cardiovascular Service and the Peripheral Vascular Clinic, Veterans Administration Hospital, Bronx, New York*

The use of vasodilator medications in advanced occlusive arterial disease is at best of limited value and its success depends upon the potential collateral circulation and the degree of local vasospasm. Although dramatic results generally cannot be expected, the contributory effect of vasodilator medications may be important and may even prevent loss of a limb with marginal arterial blood supply. Adequate and frequently optimal peripheral vasodilatation can be induced in most patients by the administration of the new anti-adrenergic agents which act predominantly on the peripheral circulation. Tolazoline[1], phenoxybenzamine[2], and the ganglionic blocking agents have been demonstrated to be the most effective of these drugs. Their use is indicated in patients who have peripheral arterial disease with a significant element of vasoconstriction, and who are able to tolerate the effects of generalized peripheral vasodilatation together with the not infrequent side-effects which are seen during treatment with all of these preparations (Table 1). In general, patients in the relatively young age group are in this category. Those of advanced age, or patients of younger ages with occlusive arterial disease in more proximal areas (including the cerebral and coronary vessels) may not tolerate these drugs when given by the usual routes. In these subjects, a method which will induce vasodilatation, well localized to the extremity which is affected by advanced arterial insufficiency, is necessary for optimal safety and effect.

The administration of vasodilator agents by the intra-arterial route offers the following theoretical advantages: 1) the effect is confined largely to the local vascular bed where it is most required, 2) there is minimal shunting of significant quantities of blood away from the more important arterial vessels in which there was either evident or occult concomitant arterial insufficiency, and 3) side effects may be avoided or minimized.

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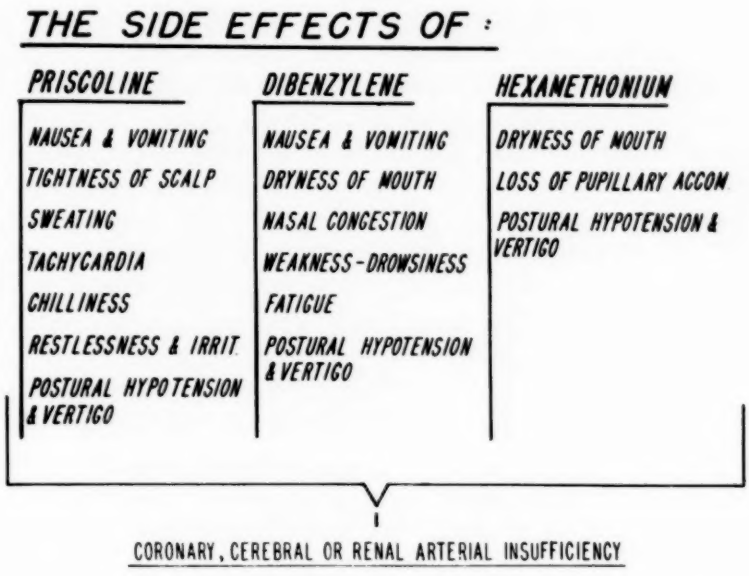
[1] Priscoline (Ciba); 2-benzyl-4,5-imidazoline hydrochloride.

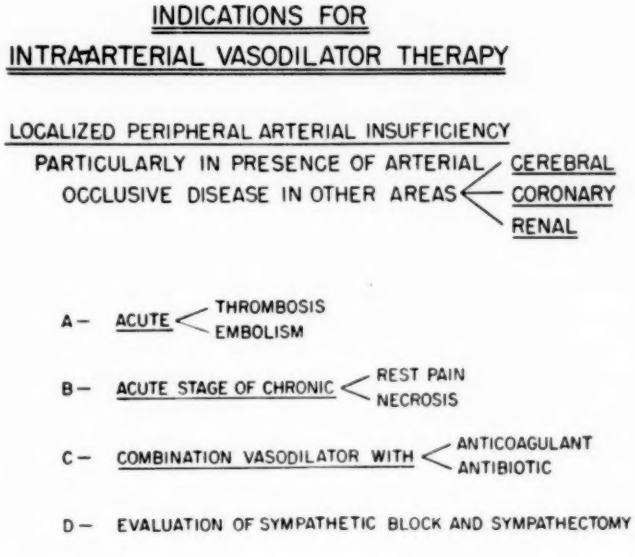
[2] Dibenzyline (Smith, Kline & French).

TABLE 1




Weissenbach and Faulong (1) in 1946 reported on the successful use of tolazoline given intra-arterially. Following this report, several investigators found this route to be effective for the administration of various vasodilator drugs (1–8). In general, tolazoline has been found to be the most effective and best tolerated agent for intra-arterial administration. Prandoni and Moser (6) recently reported on the use of this drug in large numbers of patients, with satisfactory results and relative lack of untoward side effects. They also demonstrated that the effect of intra-arterial tolazoline is well localized, and that

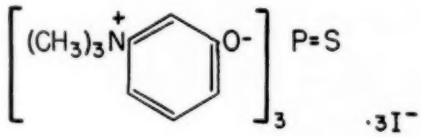
TABLE 2



temperature elevation may be obtained in normal individuals, indicative of maximum blood flow under standard conditions, and comparable to the effect of sympathetic block.

We have had considerable experience with the intra-arterial use of tolazoline, and have concluded that this is a safe and effective form of therapy. Our findings agree with those of Prandoni and Moser (6) that intra-arterial tolazoline is most effective in the treatment of ischemic rest-pain, and that it is also useful in the causalgic states, for the evaluation of lumbar paravertebral sympathetic blocks or sympathectomy. The intra-arterial route may also be used for the simultaneous administration of a vasodilator agent and an antibiotic or anti-coagulant medication. This route is primarily of value for relatively short-term therapy (Table 2).

In the past several years, we have studied and compared the effects of additional vasodilator agents given intra-arterially, in an effort to discover medica-




COMPOUND 27 MI

FIGURE 1

tions superior to tolazoline. Drugs which act directly upon vascular smooth muscle and a number of commercially available anti-adrenergic medications were tried, but in our experience none was superior to tolazoline. We have recently evaluated a new vasodilator agent which is the subject of this report. The medication is designated for investigation, 27 MI, and is described as the neutral thio-phosphoric ester of 3-hydroxyphenyltrimethyl ammonium iodide trihydrate.[3] It has the chemical formula shown in Figure 1.

Animal studies have demonstrated that this preparation has a vasodilator effect predominantly upon the peripheral arterial system when given orally and parenterally. Toxicity is extremely low. Its mode of action appears to be mainly a direct one upon peripheral vessels and there is little evidence of anti-adrenergic effect.

*Control studies.* In 10 normal males, the injection of 10 mg. of 27 MI in 1 cc. of normal saline into the femoral artery resulted in maximal temperature rises in the toes within three to fifteen minutes under basal conditions in a temperature-controlled room. Radio-sodium clearance determinations were made in these subjects, comparing the effects of 27 MI with those of tolazoline (Table 3), by the method of Freund, Wisham and Yalow (9). (Our studies were performed at a constant temperature of 68° F., which is somewhat lower than that used

[3] Supplied by the Campbell Pharmaceutical Company, courtesy of Doctor Robert Lehman.

TABLE 3

RADIOSODIUM CLEARANCE IN CALF FOLLOWING I.A. ADMINISTRATION OF PRISCOLINE AND 27 M.I.-10 NORMAL MALES

(CLEARANCE RATE = REDUCTION TO 1/2 OF INITIAL ACTIVITY)

| | RATE / MIN. | % CHANGE |
|---|---|---|
| **SKIN** | | |
| CONTROL | 11.7 | |
| PRISCOLINE-(25mgm) | 10.4 | +12.5 |
| 27 M.I. -(10mgm) | 8.1 | +44.4 |
| **MUSCLE** | | |
| CONTROL | 7.06 | |
| PRISCOLINE | 7.57 | -6.7 |
| 27 M.I. | 6.8 | +3.8 |

PREDICTABLE ERROR = ± 15%

by these authors.) There was a marked increase in the rate of clearance from the skin with 27 MI, compared to the rate with tolazoline. With 27 MI the increase was 44.4 per cent, whereas the increase with tolazoline was within the range of predictable error. Radio-sodium clearance from muscle showed no significant increase in flow with either drug. However, with 27 MI there was a consistent, slight increase in clearance rate, whereas with tolazoline there was a decrease. This may indicate that with 27 MI skin blood flow increases to a greater degree than with tolazoline, but not at the expense of muscle blood flow.

## METHODS AND MATERIAL

Sixty-three patients were treated intra-arterially with 27 MI. They were chosen for intra-arterial therapy because of signs and symptoms of advanced occlusive arterial disease in one or both lower extremities and because of advanced age, poor condition, and/or concomitant arterial insufficiency involving other areas, chiefly cardiac and cerebral (Table 4). Of these patients, 60 had arteriosclerosis obliterans as the cause of peripheral arterial insufficiency, 2 had thrombo-angiitis obliterans, and 1 had embolic occlusion of a femoral artery secondary to a myocardial infarct. This group of patients, for the most part, had received adequate trials of supportive measures and specific treatment of underlying or additional pathologic states including heart failure, diabetes and infection. The usual vasodilator medications, given by the oral route, had been simultaneously administered in some instances. Three of these patients had undergone lumbar sympathectomy previously with inadequate relief.

The 27 MI was given in doses of 5 to 20 mg., the concentration of the solution being 10 mg. per cc. of physiologic saline. A #22 or #23-gauge needle

TABLE 4

**MATERIAL**

HOSPITALIZED MALES WITH ADVANCED OCCL. ART. DIS. OF LOWER EXTREMITIES.

NUMBER – 63

AV. AGE. – 59 YRS.

ADD'L DIAG. – 1. DIABETES – 27
2. A.S.H.D. – 23
3. C.V.D. – 18

SYMPTOMS – 1. REST PAIN OR CLAUDICATION – 28
2. ULCERATION OR NECROSIS – 34

SIGNS – 1. TEMP, COLOR, TROPHIC CHANGES
2. ABSENT PULSES & OSC. BELOW KNEE
3. LITTLE OR NO RESPONSE TO 2+ VASODILATOR TESTS.

with short bevel was used, and the drug was injected directly into the femoral artery below the inguinal ligament at a rate of about 10 mg. per minute. More than 1,200 injections were given with an average of about 20 treatments per patient. Several patients received more than 50 injections in the same artery and 1 patient had more than 100 such treatments. The frequency of the injections ranged from every six hours to once a week, the average being once daily while the patient was hospitalized. Frequency of administration varied with severity of occlusive manifestations and with the response obtained; it was usually reduced progressively. Injections rarely continued for more than one month. Intra-arterial medications were always given with the patient in the supine position, and the patients remained on the table following treatment for at least fifteen minutes.

## RESULTS

Following administration of 27 MI intra-arterially, there was in almost all instances a sensation of heat, described as burning ("like pepper"). It was first felt at the site of injection and then extended progressively to the most distal portions of the feet and toes, over a period of from one to five minutes. This effect was consistent; it was different from that of tolazoline and was more striking. Usually there was rubor of the skin, extending from the site of injection to the toes, but this sign was variable, not as intense as that with tolazoline, and not related to the rise in temperature or the degree of therapeutic effectiveness. Piloerection also frequently accompanied rubor; again, it was not as consistent as with tolazoline. The sensory effect was more intense, of earlier onset and more consistent than with tolazoline, but usually was of shorter duration, lasting from one to three hours in most patients. The therapeutic effect, particularly the relief of ischemic pain, usually outlasted the original sensory effect.

*Tolerance and side effects*

In most patients, 27 MI was well tolerated. Tolerance depended largely upon the rate of administration; rapid intra-arterial injection was likely to produce side effects. Side effects included generalized flushing of the skin, tachycardia and occasional other cardiac arrhythmias, and motor weakness of the quadriceps muscle group for a short period following administration, as well as the local effect on the arterial wall (Table 5). The side effects were largely dependent on the rate of administration and the size of the dose. They could be virtually eliminated if the rate at which the drug was given did not exceed 10 mg. per minute, and the dosage was started at 5 or 10 mg. and increased progressively. The fact that no serious reactions occurred is particularly significant in this group of patients of advanced age, as they are frequently in poor general condition and often have arterial disease in other areas. The local effect of intra-arterial administration of 27 MI was less than with tolazoline, and it is our impression that local fibrosis is largely due to the repeated arterial punctures. Injection becomes somewhat difficult after a large number of treatments in the same vessel, and may be minimized by the use of small-gauge needles and by taking care to administer the medication only when the needle is shown to be well within the lumen of the artery.

*Therapeutic effect*

a. *Pain:* Ischemic rest-pain, either in the presence or absence of concomitant necrosis or ulceration, was relieved in 92 per cent of cases. The degree and duration of relief was variable. In most patients, there was complete relief of pain for several hours. In about half of the cases there was enough diminution in pain to permit reduction or discontinuance of analgesic medication for twelve or more hours. In several patients, the pain disappeared following the first

TABLE 5

**METHOD**

DOSE - 5-20 mgm.
I.A. (FEMORAL)
TOTAL # - 1,200 +
Au. 20/PT. MAX. 100 +
FREQUENCY - Au 1X DAILY

**TOLERANCE**

NO SERIOUS UNTOWARD REACTION

SIDE EFFECTS

1. GEN. FLUSH
2. TACHYCARDIA
3. MOTOR WEAKNESS
4. LOCAL FIBROSIS

injection of 27 MI and did not recur. In most of the patients, however, the drug was continued daily; thus, in them, the total duration of pain relief following a single injection could not be estimated. Relief of pain was correlated to some degree with the intensity of the burning sensation, but there was no consistent correlation with objective evidences of increased blood flow. In another series of several patients with peripheral pain due to neuropathy or to other non-circulatory factors, there was a similar pain-relieving effect. 27 MI did not appear to have a local anesthetic action, as there was no grossly discernible sensory loss. A similar effect upon pain has been noted and commented upon by Prandoni and Moser (6); they reported relief of ischemic rest-pain in about 80 per cent of patients treated with tolazoline. It is also of interest that intra-arterial tolazoline was found to relieve the pain and improve mobility in patients with severe osteoarthritis, as reported by Davison and Wolf (10). This effect of intra-arterial medications on pain appears to be unrelated to the vasodilator effect as, in most instances, its onset is too early to be explained as the result of improved blood flow and, in some instances, relief of pain has continued despite progressive necrosis.

b. *Ulceration and necrosis:* The therapeutic effect of any specific agent is extremely difficult to evaluate in this group of patients because of the large number of variables. In this group, because of the advanced nature of their disease, all forms of therapy which may be beneficial must usually be administered immediately and simultaneously; thus there is no opportunity to obtain good control observations. These patients all receive the benefit of hospital care with adequate bed rest, improved nutrition and the necessary supportive measures for other contributing pathologic conditions. Because of the extremely advanced arterial occlusive disease which is invariably present, objective tests of blood flow cannot be made frequently. Furthermore, increments of flow which might be sufficient to aid in local improvement and the saving of a limb, are usually too small to be measured by any present method. Evaluation of improvement following the use of 27 MI was therefore placed in the hands of the senior members of our clinic, and was based chiefly upon their clinical judgment. It was the opinion of these physicians that in several instances the administration of 27 MI intra-arterially aided in deferring amputation and in expediting healing and separation of necrotic areas. It is possible that the good results in this group of patients were due in some measure to relief of pain from the intra-arterial injections, and that this relief permitted the patient to tolerate his extreme degree of local arterial insufficiency and allow time for healing.

Any factor which helps to avoid amputation is of considerable value, as high amputation is invariably necessary in this group. In most instances, such amputation results in complete invalidism for these elderly, debilitated, and poor-risk patients. Intra-arterial administration of 27 MI is apparently effective and has no deleterious effects upon other areas of vascular insufficiency. It appears, therefore, to be well suited to the treatment of patients with advanced arterial disease involving the lower extremities, particularly when vas-

cular disease is present elsewhere and when the patient's general condition is considered to be somewhat precarious.

Although beneficial and safe for intra-arterial administration, the effect of 27 MI is of relatively short duration, and there are some side reactions which demand considerable care in its administration. Experience with 27 MI and other vasodilator agents given by the intra-arterial route justifies continued investigation in the search for a medication which more nearly approaches the ideal of prolonged, consistent, localized vasodilator action with minimal side effects.

## SUMMARY AND CONCLUSIONS

1. Results of intra-arterial injection of a new vasodilator drug, 27 MI, have been studied.

2. In 10 normal males used as controls, the administration of 27 MI resulted in maximum vasodilatation which occurred predominantly in the skin, but without apparent reduction in muscle blood flow.

3. In 63 patients with advanced arterial occlusive disease of the lower extremities treated with intra-arterial 27 MI, the drug was well tolerated and there were no serious side effects. The relief of pain was the most striking and consistent therapeutic effect; it occurred in 92 per cent of the cases. Healing of ulcers and necrosis appeared to be expedited by this form of treatment.

4. It is concluded that 27 MI is a safe and effective vasodilator agent for administration by the intra-arterial route.

5. This, and previous experience, indicate that intra-arterial vasodilator therapy has particular value in the treatment of patients with localized arterial occlusive disease, especially when there is concomitant vascular disease in other areas, and in the treatment of patients whose condition is considered to be too precarious for the administration of generalized vasodilators.

6. Continued investigation of vasodilator medications for administration by the intra-arterial route is indicated.

*Acknowledgment*

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# CLINICAL STUDY OF THE HYPOTENSIVE AND SEDATIVE EFFECTS OF ORAL CHLORPROMAZINE THERAPY IN CARDIOVASCULAR DISEASE*

DAVID S. WAX, M.D.† AND ARTHUR C. DEGRAFF, M.D.‡

*The Medical Service, Veterans Administration Hospital, Bronx, New York*

Chlorpromazine[1] when administered parenterally, and less commonly when given orally, is known to have a variety of effects on the cardiovascular system. These are manifested by a decrease in peripheral resistance, with hypotension and tachycardia. This report is of a study of the antihypertensive and tranquilizing effects of orally administered chlorpromazine in patients with hypertension and in patients with the anginal syndrome and superimposed cardiac neurosis.

Chlorpromazine was first used clinically by Laborit, Huguenard and Alluaume (1, 2) in 1951. They described its sympatholytic and anticholinergic ganglionic blocking actions and postulated a central action in an area between the cerebral cortex and the diencephalon. Courvoisier and associates (3) also described this action on the parasympathetic and sympathetic nervous systems, as well as the central sedative, hypothermic, anti-emetic and metabolic depressant properties of chlorpromazine. Courvoisier *et al.* demonstrated that chlorpromazine has adrenolytic properties, by blocking the pressor action of intravenous epinephrine and norepinephrine and causing a hypotensive effect similar to that seen with Dibenamine when large doses were used. They also found that chlorpromazine has an antifibrillatory effect. This anti-adrenergic action was confirmed by Finkelstein *et al.* (4). They demonstrated that chlorpromazine prevented the fibrillatory effect of epinephrine, *l*-norepinephrine and N-isopropyl-norepinephrine on the papillary muscle of the cat. Melville (5), using pentobarbitalized and morphinized cats and dogs, was able to prevent the extrasystolic and fibrillatory action of epinephrine and norepinephrine, particularly after chloroform anesthesia, by means of chlorpromazine. Foster *et al.* (6), using chlorpromazine parenterally, observed a diminution in the pressor effect of norepinephrine and a reversal of the action of epinephrine on the vessels of the hand.

In addition to its ganglionic blocking action and its anti-adrenergic properties, chlorpromazine has been demonstrated to have a direct effect on blood vessels. Courvoisier *et al.* (3) described vasodilatation following both intravenous and intra-arterial administration of the drug. Foster *et al.* (6), following intravenous injection of chlorpromazine, demonstrated by venous occlusion plethysmography,

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† Research Fellow in Cardiology, Veterans Administration Hospital, Bronx, New York. Supported by the Committee for the Promotion of Medical Research, Inc.

‡ Senior Consultant in Cardiology, Veterans Administration Hospital, Bronx, New York.

[1] Chlorpromazine (Thorazine®) was supplied by Smith, Kline and French Laboratories, Philadelphia, Pa.

an increased blood flow in the hands, forearms and calves, as well as vasodilatation. Similar results by intra-arterial administration were obtained after blocking of the brachial plexus. They concluded that chlorpromazine is a powerful vasodilator of the blood vessels of the limbs, as a result of both central and peripheral actions.

Foster *et al.* furthermore, decreased the cold pressor response by means of chlorpromazine; and Laborit abolished acrocyanosis by its use. Dobkin, Gilbert and Lamoureau (7), following intravenous administration of the drug, observed a rise in the temperature of frostbitten limbs, and suggested that chlorpromazine might be of value as an agent in the diagnosis and treatment of peripheral vascular diseases.

Courvoisier *et al.* (3) and Melville (5) reported an increased coronary blood flow following chlorpromazine administration in experimental animals.

The hypotensive action of chlorpromazine was first noted by Laborit and co-workers (1, 2) following its parenteral administration; they noted vascular paralysis when it was used concomitantly with pronestyl. Moyer *et al.* (8), by repeated intravenous injection of chlorpromazine into anesthetized dogs, observed tachycardia and a fall in blood pressure, which they attributed to vasodilatation with decreased peripheral resistance. Dobkin *et al.* (7), upon administration of the drug to patients, noted a rise in pulse rate initially; there was marked tachycardia and orthostatic hypotension, if the patients were put in the upright position. Foster *et al.* (6) also induced in human volunteers (anesthetized or awake) an increased pulse rate and a fall in systolic and diastolic pressures. Friend and Cummins (9), following intramuscular administrations of chlorpromazine, noted side effects that included drying of the mouth, sedation, transient faintness, flushing of the face, palpitation and tachycardia.

In summary, chlorpromazine, parenterally administered, has cardiovascular effects which appear to be mediated 1) through the central nervous system (13), by inhibiting neuronic activity in the cortical and hypothalamic areas, and 2) through the sympathetic nervous system, by sympatholytic and anticholinergic ganglion blocking effects peripherally. The drug is also anti-adrenergic and has a direct effect on blood vessels, including that of coronary vasodilatation. The following study concerns the cardiovascular effects of oral chlorpromazine in a group of hypertensive patients and in patients with the anginal syndrome.

## METHOD

Seven patients with hypertension and 4 with the anginal syndrome and superimposed cardiac neurosis were selected at random and studied by means of a "double blind" technic. After an initial period of observation and determination of base-line levels, an amytal test or a cold-pressor test was performed in the hypertensive patients; an electrocardiogram was taken; the heart size was determined by x-ray; the funduscopic findings were classified; and hepatic, renal and hematopoietic functions were studied. The patients were started on doses of chlorpromazine or placebo ranging from 30 to 75 mg. per day, increased in all but one instance to a maximum of 150 mg. per day. The patients

were maintained on each drug for a period of from two to eight weeks. In the hypertensive group, the blood pressure determinations were made daily at comparable times. In the anginal syndrome group, the sedimentation rate was followed and the patients kept a daily diary of their symptoms. This diary classified their pain as to time of occurrence, activity at the onset of pain, type, location and severity of the pain, its radiation, and the medication taken for relief. A uniform dosage of 75 mg. daily was used in this group. In both groups of patients, before an increase in dosage or at the time of drug interchange, the following functions were studied: *Hematopoietic*—complete blood count. *Hepatic*—bromsulfalein excretion; serum levels of bilirubin, alkaline phosphatase, cholesterol, total protein; serum albumin/globulin ratio, cephalin flocculation and thymol turbidity. *Renal:* Urinalysis, blood urea nitrogen level, urinary phenolsulfonphthalein concentration test, and urea clearance. The complete series of renal and hepatic tests was not performed in every patient at each change in dosage. Specific tests, however, were serially followed in each patient. A complete study was performed at the time of drug changes and at the completion of the study.

## RESULTS

### *A. The Hypertensive Group (7 patients; Table 1)*

One patient (No. 4) experienced mild "grogginess" and "fatigue" while receiving chlorpromazine, but not while receiving the placebo. This patient's blood pressure remained the same while he received chlorpromazine, but in the control period, during an acute renal infection, the blood pressure fell to normotensive levels. A second patient (No. 5), while receiving the placebo after an initial trial of chlorpromazine, had a cerebrovascular accident, without change in his blood pressure. A third patient (No. 7), while receiving chlorpromazine, showed a cephalin flocculation of 2 plus during an episode of gastro-intestinal bleeding. Previously, while receiving placebo, this patient had had a transitory lymphocytosis. In the patients in whom cold-pressor tests were performed, there were no changes either with the placebo or with chlorpromazine. Potentiation of the amytal test did not occur after chlorpromazine. The tests performed showed no abnormalities of renal, hepatic or hematopoietic function attributable to chlorpromazine, and no significant changes in the blood pressure were observed.

### *B. The Anginal-Syndrome Group (4 patients; Table 2)*

Two of the patients obtained initial subjective relief, noted as a decrease in the frequency of "anginal" attacks, although the diary of one revealed no changes in the severity or frequency of "anginal" episodes. One of these patients was started on chlorpromazine and the other on placebo. Both patients, when the drugs were interchanged without their knowledge, had no change in symptomatology. A third patient experienced no change during administration of chlorpromazine or of placebo, either in anginal symptoms or in his highly labile

TABLE 1

*Hypertensive Group*

| Patient, Age, Sex, Diagnosis | Range of Blood Pressure (mm. Hg) | | | Drug Admin'd Initially | Max. Daily Dose of Chlorpromazine (mg.) | Duration (wks.) | | No. of Exchanges of Drug & Placebo | Toxicity | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Placebo | Drug | | | Drug | Placebo | | | |
| 1. A. F., 57, male, HCVD,* CHF, mild diabetes | 160/110<br>210/140 | 150/90<br>200/130 | 140/100<br>210/130 | Chlorpromazine | 150 | 4 | 2+ | 1 | None | No change in amytal tests |
| 2. J. N., 66, male, HCVD, AS-PVD | 152/90<br>180/118 | 110/72<br>196/120 | 140/90<br>170/110 | Chlorpromazine | 30 | 2+ | 4 | 1 | None | No change in cold-pressor tests |
| 3. A. McN, 41, male, essential hypertension | 120/70<br>170/115 | 120/80<br>180/120 | 140/80<br>170/110 | Placebo | 150 | 3+ | 4 | 1 | None | No change in cold-pressor tests |
| 4. L. D., 29, male, HCVD, chronic pyelonephritis | 110/72<br>170/120 | 150/95<br>170/125 | 150/90<br>150/110 | Placebo | 150 | 3 | 3 | 1 | None | B.P. 110/72 during control period associated with an acute febrile episode. Lowest reading otherwise, 140/100. No change in cold-pressor test |
| 5. D. W., 55, male, HCVD, pyelonephritis | 150/96<br>186/120 | 152/100<br>180/136 | 160/90<br>240/160 | Chlorpromazine | 150 | 8 | 2 | 1 | None | B.P. on admission 240/140. Test discontinued. Pt. had a cerebrovascular accident. Cold-pressor, & amytal tests not made |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 6. F. M., 65, male, HCVD, ASHD | 120/80<br>190/120 | 110/80<br>160/90 | 116/80<br>132/94 | Placebo | 150 | 2+ | 2 | 1 | None | Mildly positive results with cold-pressor test remained unchanged. Positive results with amytal test. |
| 7. R. H., 61, male, HCVD, chronic renal disease, type undet'd. | 140/90<br>210/120 | 130/80<br>160/120 | 130/88<br>190/140 | Placebo | 150 | 2 | 4 | 1 | Ceph. flocc. 2+ with chlorpromazine | Positive results with amytal test. Cold-pressor test not made after chlorpromazine. Ceph. flocc. 2+, during G.I. bleeding |

* HCVD = Hypertensive cardiovascular disease.
CHF = Congestive heart failure.
AS—PVD = Arteriosclerotic peripheral vascular disease.
ASHD = Arteriosclerotic heart disease.

TABLE 2

*Angina Group*

| Patient, Age, Sex, Diagnosis | No. of "Angina Attacks" Per Day | | | Drug Admin'd Initially | Max. Daily Dose of Chlorpromazine (mg.) | Duration (wks.) | | No. of Exchanges of Drug & Placebo | Toxicity | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Placebo | Drug | | | Drug | Placebo | | | |
| 1. F. S., 56, male, ASHD,* anxiety neurosis | 3–4 | 3–4 | 3–4 | Placebo | 75 | 4 | 2+ | 1 | None | Patient subjectively improved on drug and placebo. Diary: no change; severity of pain unchanged |
| 2. M. B., 57, male, ASHD, psychoneurosis | 1–2 | 1 per week | 1–2 | Chlorpromazine | 75 | 2+ | 4 | 1 | Control BSP 2%; with chlorpromazine, BSP 13% | Subjectively, initial beneficial effect from both drugs. Diary: fewer attacks with placebo; severity of attacks unchanged |
| 3. H. G., 63, male, ASHD, angina, severe psychoneurosis | 1–2 | 2–3 | 1–3 | Placebo | 75 | 4 | 2+ | 1 | Eosinophilia with chlorpromazine | Patient relieved by placebo prior to study. Diary: no real change |
| 4. R. G., 38, male, ASHD, psychoneurosis | 3–4 | "Marked relief" | No change | Chlorpromazine | | 2 | — | 2 | None | Relief with placebo. No change with chlorpromazine; severity of attacks unchanged |
| | | | | Placebo | 75 | — | 2 | | | |
| | | | No change | Chlorpromazine | | 2 | — | | | |

* ASHD = Arteriosclerotic heart disease.

emotional state. The fourth patient obtained relief from the placebo. When, without his knowledge, chlorpromazine was substituted, symptoms recurred; when the placebo was re-instituted, symptoms were again relieved. Except for eosinophilia in one patient and a bromsulfalein retention of 13 per cent in another patient, there were no abnormalities in the tests of hepatic, renal or hematopoietic function while the patients were receiving chlorpromazine.

## DISCUSSION

The use of the "double blind" test in evaluation of a new drug, particularly one that has tranquilizing effects, is essential. This is particularly necessary when the drug is administered to patients with hypertension and angina, in whom symptoms are associated with, partly caused by, or modified by, psychic factors. The response of patients with angina to placebo therapy is well known. The subjects in this study were selected at random and started alternately on either placebo or chlorpromazine. This procedure was carried out in order to obviate the tendency of the patient to experience a greater effect from the second drug. Minor differences in effect between the two drugs were judged to be of little significance. In the hypertensive group, no differences were noted in blood pressures or subjective symptoms. Significantly, during the control period, several of these patients had a marked decrease in blood pressure compared with the admission level. In the anginal-syndrome group, relief was obtained within a day of onset of therapy, whether chlorpromazine or placebo was given. In some instances, the degree of improvement seemed to be less as the novelty of therapy waned.

The maximum dosage was 150 mg. of chlorpromazine per day, in the hypertensive group and 75 mg. per day in the angina group. This dosage was not exceeded because of reports in the literature of jaundice occurring more commonly with larger doses. It is possible that higher oral dosage is necessary in order to obtain the hypotensive effects reported with parenteral administration; higher doses, orally, are known to have a sedative effect in agitated psychotic patients. The drugs were given for intervals of from two to eight weeks, with an average of about three weeks. Again, it is possible that prolonged administration is necessary in order to obtain a hypotensive effect; however, the blood pressure readings during the placebo periods following chlorpromazine administration were not different from the readings observed when placebo was given initially. During chlorpromazine administration, the lower blood pressure readings were not necessarily obtained toward the end of the course of therapy. Indeed, there was no pattern to the rise and fall of the blood pressure, which was unpredictable from day to day. In view of the much discussed "chlorpromazine jaundice," it is noteworthy that in only 1 patient was there a record of an abnormality in liver function. The occurrence of a moderate eosinophilia in 1 patient while receiving chlorpromazine is unexplained.

## SUMMARY

Chlorpromazine, parenterally administered, causes cardiovascular changes which include hypotension, generalized vasodilatation with decreased peripheral

resistance, tachycardia, and coronary vasodilatation. The drug inhibits neuronic activity in the cortical and hypothalamic areas centrally, is a sympatholytic and parasympatholytic ganglionic blocking agent peripherally, is adrenolytic, and exerts a direct effect on peripheral blood vessels.

A double blind test was performed with 7 hypertensive patients and 4 with the anginal syndrome and superimposed cardiac neurosis. They were selected at random, in order to determine effectiveness of chlorpromazine orally in cardiovascular disease. In both groups, base-line observations were obtained during a control period and tests of hepatic, renal and hematopoietic function were performed at the times of changing either dosages or drugs. In the hypertensive group, results of cold-pressor and amytal tests and daily blood pressure readings at comparable times were used as criteria of the effect. Patients in the anginal-syndrome group kept daily diaries of their attacks of chest pain; their sedimentation rates and electrocardiograms were followed in the laboratory. In the hypertensive group, the maximum dosage was 150 mg. per day, and in the anginal-syndrome group, 75 mg. per day. The overall length of drug administration varied from six to ten weeks.

There were no significant changes in blood pressure, potentiation of the amytal test or diminution in the response to the cold-pressor test during control, placebo or chlorpromazine periods. The effect in the anginal-syndrome group was similar to that observed with any placebo. There were no significant changes in hepatic, renal or hematopoietic function during chlorpromazine administration.

## CONCLUSIONS

1. Chlorpromazine, given orally in a "double blind" test in dosages up to 150 mg. per day, for periods of from two to eight weeks, to 7 hypertensive patients, failed to lower blood pressure significantly or to block the positive response to the cold-pressor test.

2. Chlorpromazine, given orally in a "double blind" test in doses of 75 mg. per day for periods of from two to four weeks to 4 patients with the anginal syndrome and psychogenic symptoms, had no effect other than that seen with placebo therapy.

3. Chlorpromazine caused no significant toxic disturbances in hepatic, renal or hematopoietic functions in these patients.

4. Because of the small size of the groups studied, no further conclusions can be drawn.

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# EFFECT OF RESERPINE, WITH OR WITHOUT HORMONAL THERAPY, UPON THE FEMALE CLIMACTERIC*†

HERBERT S. KUPPERMAN, PH.D., M.D., MEYER H. G. BLATT, M.D., LEE F. VOSBURGH, M.D., JOHN CANIS, M.D. AND BENJAMIN WETCHLER, M.D.

*Departments of Therapeutics and of Obstetrics and Gynecology, New York University-Bellevue Medical Center, and the Obstetrical and Gynecological Service (III Division), Bellevue Hospital, New York, N. Y.*

Adequate control of the female climacteric may be achieved by psychotherapy, sedation and administration of various hormones, both singly or in combination (1). Of the therapeutic procedures involving drug therapy, the use of estrogens alone or together with androgens has been shown to be the most effective means of treating most climacteric patients with significant symptoms (2). However, due to some undesirable side effects that occasionally may be noted after estrogenic therapy and the purported neoplastic propensities, the use of estrogens has been condemned by some physicians (3–4). This is especially true in patients in whom there is a family or past history of genital neoplasm. In an attempt to diminish and remove these objections, sedation therapy depending primarily upon the use of barbiturates alone or in combination with small doses of estrogens has been suggested. It was thought that the latter combination would achieve adequate control of the climacteric syndrome without administering objectionable doses of estrogen. However, the barbiturates themselves may have an added undesirable side effect, in that there is always the potentiality of untoward accumulation and possible addiction to the drug. Therefore we have attempted to evaluate the effect of reserpine, the alkaloid originally prepared from Rauwolfia serpentina, in a number of well-documented female patients with the climacteric syndrome.

Crude extracts and purified alkaloids of Rauwolfia serpentina have been known to medicine in India for some time (5–6). These preparations have been used in India with varying degrees of success in the control of certain mental aberrations characterized by anxiety or apprehension, and in some patients who were in psychotic phases of their illness (7). The drug has also been used in India in the treatment of epilepsy. It has several interesting properties, the main pharmacologic action being attributed to central nervous system effects associated with sedation and minimal hypnosis.

## METHOD

In an attempt to evaluate reserpine properly in the climacteric patient, various combinations of the purified alkaloid preparation with different hormones

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TABLE 1

## MENOPAUSAL INDEX

| SYMPTOMS | FACTOR | SEVERITY | NUMERICAL CONVERSION |
|---|---|---|---|
| 1. VASOMOTOR | 4 | + = 3 | 12 |
| 2. PARESTHESIA | 2 | M = 2 | 4 |
| 3. INSOMNIA | 2 | M = 2 | 4 |
| 4. NERVOUSNESS | 2 | S = 1 | 2 |
| 5. MELANCHOLIA | 1 | S = 1 | 1 |
| 6. VERTIGO | 1 | 0 = 0 | 0 |
| 7. WEAKNESS (FATIGUE) | 1 | M = 2 | 2 |
| 8. ARTHRALGIA AND MYALGIA | 1 | S = 1 | 1 |
| 9. HEADACHES | 1 | + = 3 | 3 |
| 10. PALPITATION | 1 | M = 2 | 2 |
| 11. FORMICATION | 1 | S = 1 | 1 |
| MENOPAUSAL INDEX (SUM)..... | | | 32 |

CODE

0 - NONE = 0 M - MODERATE = 2

S - SLIGHT = 1 + - MARKED = 3

were studied by the standard procedure used in this clinic. The preparations[1] were administered to patients who complained of either the natural or surgical climacteric, including many whose response to standard preparations and placebos had been well characterized. The patients were evaluated according to the technique previously described (2), which involves the blind testing of the drug concerned. In this method, neither the investigator nor the patient knows what preparation is being administered, and in the climacteric it has been found to be a reliable and completely objective means of evaluation of endocrine and non-endocrine preparations (2).

The patient is interrogated with respect to the eleven most common complaints noted during the climacteric. The list of such symptoms is shown in Table 1. A "menopausal index" is then derived, which characterizes the severity of the complaints. Alterations in the menopausal index are utilized as a measure

[1] Preparation 1 was supplied by Dr. Robert Broh-Kohn of Bard Pharmaceutical Co.

Preparations 2 (Estinyl) and 4 (Gynetone) were supplied by Dr. Norman Heminway of Schering Corporation.

Preparation 3 (Premarin) was supplied by Dr. John Jewell of Ayerst, McKenna & Harrison.

Preparation 6 was supplied by Dr. C. O'Donovan of the Upjohn Co.

Preparations 7–11 were supplied by Dr. F. Travers of Ciba Pharmaceutical Products, Inc.

Preparation 12 (Bellergal) was supplied by Mr. Kenneth Ericson of Sandoz Pharmaceuticals.

of the effectiveness of therapy. Of the eleven symptoms studied, the first four are considered by far the most important, *viz.*, vasomotor disturbances, insomnia, nervousness and paresthesia. In addition, melancholia, vertigo, weakness or fatigue, arthralgia-myalgia, headaches, palpitation and formication are listed. Since the first four symptoms are most common and of greatest consequence in the climacteric syndrome, they are given greater emphasis in formulating the menopausal index. The subjective findings as noted in the patients, are evaluated and converted to a numerical figure based upon the severity and extent of their complaints. It is the sum of the individual numbers noted for these patients that constitutes the menopausal index and thus serves as a guide to the severity of the climacteric disturbance.

The vasomotor symptoms occur comparatively late as a manifestation of the climacteric syndrome and may appear only after the menopause has intervened. They are, however, by far the most common complaint, and the one which leads the patient to seek advice from the physician. Because of their importance the vasomotor symptoms are given a weighted value, arbitrarily designated by the factor 4. This means that in the process of numerical conversion, the initial value for the severity of these symptoms in a given patient is multiplied by 4. The remaining three of the first four symptoms—insomnia, nervousness and paresthesia—although outweighing the subsequently listed symptom complexes, are not of as great clinical significance as the vasomotor disturbances, and thus are given the factor 2 as a multiplier in the numerical conversion of the severity of the patient's symptoms. The remaining symptoms have a factor of 1. The numerical conversion employed in the menopausal index is secured by using the foregoing factors in multiplying the numerical equivalents of the severity of the symptoms noted in a patient upon interrogation. The numerical severity of the syndrome is somewhat arbitrarily obtained. The mathematics involved are comparatively simple and the conversion is based upon the following: If the patient does not complain of any significant symptoms in the particular category about which she is being interrogated, a "0" is placed in the "severity" column on the chart, the arithmetic conversion being zero. However, if the patient complains of slight symptoms, the symbol "S" is placed in the column, and the arithmetic conversion arbitrarily is 1. If the patient complains of moderate symptoms, the letter "M" is placed in the column; the numerical conversion of this degree of severity is 2. Finally, if the patient complains of "marked" symptoms, a "plus" sign is placed in the column, and the arithmetic conversion of "plus" is 3. The severity of the symptom is then multiplied by the factor, as previously outlined. An arbitrary example is presented in Table 1, showing how the data are obtained and their significance. It may be seen that the vasomotor symptoms can alter the menopausal index to such a degree that they may outweigh all other complaints. The importance given to the vasomotor symptoms in the female climacteric is justifiable, since many of these women can tolerate most of their discomfort except for the vasomotor symptoms, which are characterized by the patient as "hot flushes."

Based upon the sum total of the numerical conversions, the menopausal

index is derived. The effectiveness of therapy is judged by the adequate reduction of the numerical index to a figure usually below 10. With severe menopausal symptoms the index would be approximately 35; with moderately severe symptoms it would be between 20 and 35; and with comparatively mild symptoms it would be in the range of 15.

In order to ascertain the validity of the observations with drug therapy, placebos were used widely throughout this study. If the patient showed adequate

TABLE 2

THE EFFECT OF RESERPINE ALONE AND IN COMBINATION WITH ESTROGENIC COMPOUNDS UPON THE MENOPAUSAL SYNDROME

| PREPARATION | DAILY DOSE (MGM.) | NUMBER OF PATIENTS | RESPONSE | | |
|---|---|---|---|---|---|
| | | | EXCELLENT | MODERATE | POOR |
| RESERPINE<br>AMPHETAMINE SULFATE<br>HOMATROPINE METHYL BROMIDE | 0.25<br>0.5<br>5.0 } B.I.D. | 43 | 6 | 9 | 28 |
| ETHINYL ESTRADIOL | 0.05 | 165 | 104 | 39 | 22 |
| CONJUGATED ESTROGENS (EQUINE) | 1.25 | 229 | 160 | 49 | 20 |
| ETHINYL ESTRADIOL<br>METHYL TESTOSTERONE (DELAYED ACTION) | 0.02<br>5.0 | 59 | 25 | 22 | 12 |
| STILBESTROL | 0.1 } T.I.D. | 49 | 15 | 15 | 19 |
| RESERPINE<br>DIETHYLSTILBESTROL<br>METHYL TESTOSTERONE | 0.25<br>0.25<br>5.0 | 32 | 9 | 11 | 12 |
| RESERPINE<br>ETHINYL ESTRADIOL<br>METHYL TESTOSTERONE | 0.1<br>0.01<br>2.5 } B.I.D. | 20 | 8 | 10 | 2 |
| RESERPINE<br>ETHINYL ESTRADIOL | 0.1<br>0.02 } B.I.D. | 43 | 12 | 11 | 20 |
| RESERPINE<br>ETHINYL ESTRADIOL | 0.2<br>0.02 } B.I.D. | 25 | 12 | 3 | 10 |
| RESERPINE<br>METHYL TESTOSTERONE | 0.2<br>5.0 } B.I.D. | 35 | 15 | 9 | 11 |
| RESERPINE<br>ETHINYL ESTRADIOL<br>METHYL TESTOSTERONE | 0.2<br>0.02<br>5.0 } B.I.D. | 56 | 29 | 10 | 17 |
| PHENOBARBITAL<br>BELLAFOLINE<br>ERGOTAMINE TARTRATE | 20<br>0.1<br>0.3 } T.I.D. | 45 | 11 | 14 | 20 |
| PHENOBARBITAL | 15 } T.I.D. | 100 | 23 | 21 | 56 |
| PLACEBO | 1 TABLET B.I.D. OR Q.D. | 493 | 99 | 82 | 312 |

response to placebo therapy, there was little purpose in subjecting her to comparative evaluation of different compounds for the treatment of the climacteric.

## RESULTS

Using the technic outlined, several different preparations containing reserpine and certain steroid hormones were administered to patients over a period of four to six weeks. Depending upon the dose administered, the medication was usually taken twice to three times per day. The doses, the types of preparation, and their various components are listed in Table 2. In Figure 1 is a graphic presentation of the results, showing the percentages of patients responding. Although vaginal smears were taken initially for study, they were soon discontinued, as there was poor correlation between the clinical effects of therapy and the response of the vaginal smear, especially when non-estrogenic compounds were used.

Two standard estrogenic preparations were employed in order to show what response could be anticipated with adequate estrogenic dosage, and to establish a baseline for evaluating the clinical response to reserpine. These estrogens were ethinyl estradiol administered in a dosage of .05 mg. per day and conjugated estrogens (equine) in a dosage of 1.25 mg. per day, and the data indicate that they had an overall effectiveness of between 70 and 90 per cent in most of the patients. In an attempt to determine the relative effectiveness of reserpine, it was

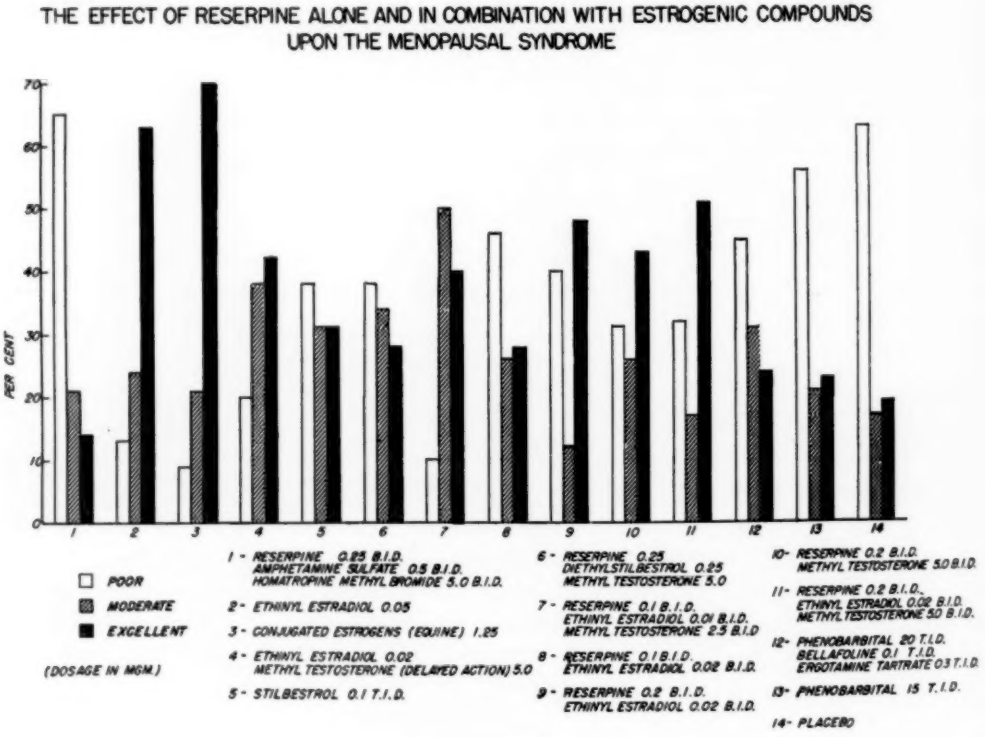
THE EFFECT OF RESERPINE ALONE AND IN COMBINATION WITH ESTROGENIC COMPOUNDS UPON THE MENOPAUSAL SYNDROME
PER CENT
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
POOR
MODERATE
EXCELLENT
(DOSAGE IN MGM.)
1 - RESERPINE 0.25 B.I.D. AMPHETAMINE SULFATE 0.5 B.I.D. HOMATROPINE METHYL BROMIDE 5.0 B.I.D.
2 - ETHINYL ESTRADIOL 0.05
3 - CONJUGATED ESTROGENS (EQUINE) 1.25
4 - ETHINYL ESTRADIOL 0.02 METHYL TESTOSTERONE (DELAYED ACTION) 5.0
5 - STILBESTROL 0.1 T.I.D.
6 - RESERPINE 0.25 DIETHYLSTILBESTROL 0.25 METHYL TESTOSTERONE 5.0
7 - RESERPINE 0.1 B.I.D. ETHINYL ESTRADIOL 0.01 B.I.D. METHYL TESTOSTERONE 2.5 B.I.D.
8 - RESERPINE 0.1 B.I.D. ETHINYL ESTRADIOL 0.02 B.I.D.
9 - RESERPINE 0.2 B.I.D. ETHINYL ESTRADIOL 0.02 B.I.D.
10 - RESERPINE 0.2 B.I.D. METHYL TESTOSTERONE 5.0 B.I.D.
11 - RESERPINE 0.2 B.I.D., ETHINYL ESTRADIOL 0.02 B.I.D. METHYL TESTOSTERONE 5.0 B.I.D.
12 - PHENOBARBITAL 20 T.I.D. BELLAFOLINE 0.1 T.I.D. ERGOTAMINE TARTRATE 0.3 T.I.D.
13 - PHENOBARBITAL 15 T.I.D.
14 - PLACEBO

FIGURE 1

decided to administer a dose of steroid hormone somewhat less than that usually required for adequate control when a steroid hormone alone was used.

On the basis of the dosage employed, our results indicate that during therapy with a combination of reserpine and a steroid sex hormone there is no synergism between the alkaloid and the estrogen in the control of the climacteric syndrome. The overall effectiveness of reserpine plus estrogen, on the basis of activity of the steroid alone, is not comparable to the control achieved with the standard estrogen. Moreover, the combination of reserpine with estrogen presents an inflexible preparation, the dosage of which can not be adjusted to the requirements of the patient's condition, and the contents of which can not be regulated when an untoward reaction to one component or the other is noted. In view of the inflexibility of such combinations, their administration is not advisable in the therapy of the menopausal patient, contrary to the opinion of some other observers (8).

Untoward side effects due to reserpine were noted occasionally in our patients, and at times they appeared to aggravate the severity of all symptoms. The undesirable side effects observed in some of the patients were: 1) excessive sedation, 2) nasal stuffiness, 3) bizarre dreams and nightmares, and 4) a Parkinsonian-like syndrome.

The side effect which was the most frequent cause of complaint in our series was nasal stuffiness. This can be a source of great discomfort to the patient and can accentuate some of the other symptoms. It occurred despite the comparatively small dosage of reserpine—0.1 to 0.2 mg. twice a day. Under such circumstances, the use of a fixed combination makes it impossible to alter therapy by removing the offending drug unless the preparation be discontinued entirely.

Combinations of reserpine with other drugs, such as the amphetamine derivatives or homatropine methyl bromide, did not result in any appreciable reduction in the primary toxicity due to the reserpine alkaloid itself. Such combinations were used in an attempt to diminish the excessive sedation and/or the nasal stuffiness that were occasionally noted with the other preparations.

During the ingestion of the various reserpine preparations the patients were interrogated concerning any alteration in libido. It was concluded that reserpine had no discernible effect upon libido. Some patients thought there might have been an increase; others thought there was a decrease; and many observed no change. Those who noted a change were checked with placebo therapy; the results were similar, indicating that the reserpine therapy had little or no effect. Moreover, in our experience, reserpine therapy does not have a noticeable effect in patients with manifestations of disturbed libido (8).

## SUMMARY

A study of the effect of reserpine alone and in combination with various sex steroid preparations in the climacteric syndrome was made in a series of well-documented menopausal patients. Reserpine did not enhance the effectiveness of the sex steroid in controlling symptoms. The combination of reserpine and sex hormones presents an inflexible preparation that does not permit the physician

to adjust the dosage of any component in the event of an untoward effect. The use of reserpine and of sex hormones simultaneously in separate tablets may occasionally be of some value in the treatment of the climacteric syndrome. The combination of these drugs in a single tablet is considered undesirable.

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# CLINICAL ASPECTS OF THE EVALUATION OF ANALGESICS*

R. W. HOUDE, M.D. AND S. L. WALLENSTEIN, M.S.

*Division of Clinical Investigation, Sloan Kettering Institute; Department of Medicine, Sloan-Kettering Division of Cornell University Medical College; Medical Service, Memorial Center for Cancer and Allied Diseases, New York, N. Y.*

The supreme test of the worth of an analgesic is its ability to allay suffering in the clinical situation, when employed to relieve pain due to disease or trauma. The goal of the investigator in this field is to be able to predict the clinical usefulness of an analgesic on the basis of his necessarily limited research data.

Because of the subjective nature of pain and our present incomplete understanding of its precise mechanisms, the detection of the presence, absence, or modification of pain, and consequently the ability to evaluate analgesic drugs, must finally be based on observations in man. In this era of rapid progress in the development of new synthetic organic compounds, it is not possible or even desirable to screen all new drugs for potential analgesic power in man. There is thus a need for laboratory animal procedures which yield results which can be correlated well with those in man. Unfortunately, with due humility, it must be admitted that the clinical yardstick has not in itself been a precise one, for the medical literature on analgesic agents is replete with conflicting reports of the efficacy of individual drugs and it is apparent that there is still considerable divergence of opinion as to how to approach the problem of testing them.

The history of analgesic testing as it is understood today dates back only to the latter part of the last century, following the isolation of active alkaloids from crude natural plant materials and the birth of the synthetic organic chemical industry. However, the earliest records of man recount legends of the effects of the poppy, mandragora, hemp and henbane, the narcotic properties of which gave comfort to all sufferers (1). We can surmise that primitive man in his persistent search for food accidently discovered that some plants had the power to obtund pain. Whether or not the true significance of these discoveries was ever appreciated, it became lost in the welter of superstition of early man's concept of his being, his surroundings and the nature of his pain. These remedies did, however, assume symbolic significance and persisted in his mystic rites; indeed, their efficacy was probably considerably enhanced by suggestion during the ritual, whether merely to ward off evil demons or to appease an offended deity. Although these early religious beliefs have been replaced, modern man is no less prone to give symbolic significance to his pain, his relationship with his physician and the mere ritual of receiving medication for relief of his suffering. The extent to which these factors influence the effectiveness of any particular medication is often not fully appreciated.

The 19th century also marked the transition from the traditional theory of

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the philosophers that pain was solely an emotional process, to the concept of pain as a specific sensation (2). The contributions to the understanding of the physiology of pain pioneered by Schiff, Blix, Goldscheider, Von Frey and Head and more recently by Adrian, Ranson, Woolard, Weddell, Wolff, Tower and many others have also paved the way for the transfer of the problem of the evaluation of analgesic drugs to the laboratory. A variety of ingenuous methods of evoking painful sensation have been devised to test analgesics by their effect on the pain threshold (3). These, for the most part, are methods that have been adapted for use in laboratory animals. Perhaps the best known of such methods is the one evolved by Hardy, Wolff and Goodell (4), who demonstrated not only that most common analgesics raise the pain threshold to radiant-heat stimuli but that some also have the additional effect of dissociating pain perception from reaction to pain, and that in the presence of pre-existing pain the latter is the dominant, or only effect observed (5). They also demonstrated that suggestion or distraction may likewise raise the pain threshold and that this effect can be as great or greater than that of the milder analgesics (6). If, however, the major effect of analgesic drugs is to alter the reaction to pre-existing pain, we may expect discrepancies between reports of analgesia based on pain-threshold measurements and on estimates of clinically-occurring pain. Factors, such as anxiety, are operant in a different degree in the clinical setting and may well alter the psychic modification of painful stimuli. Some investigators in this field, notably Beecher, have come to the conclusion that experimental methods "are not suitable to the purpose put and that appraisal of real analgesic power must be based upon the capacity of the agent to relieve 'natural' pain, pain that is a consequence of disease or trauma." (7).

## DESIGN FOR TESTING

The main advantage of the laboratory approach is that the experimental situation can be more easily manipulated by the investigator and thus the variables may be under better control. Although the clinical setting is admittedly less flexible, there has been a growing awareness in recent years of some of the variables that may influence the results, and the fact that these can and should be controlled in order to obtain reliable information. There are five major variables concerned in the clinical evaluation of analgesics: 1) the drug, 2) the patient, 3) the setting, 4) the observer, and 5) the investigator's operational concepts.

*The drug.* In the design of a clinical study one should attempt either to eliminate or equate all variables other than that which is to be measured. In the clinical evaluation of analgesic drugs, relief of pain is the goal, and by the very definition of an analgesic, it must be ascertained that the evaluation is not based on measuring specific therapeutic effects on the basic disease process. Batterman has rightfully pointed out "the inability of the patient to distinguish between a palliative or symptomatic relief and a cure of the painful condition" (8). We must also be certain that we are indeed measuring the analgesic effect of the drug and not merely the analgesic effect of the ritual implied in the administration of the drug. It is impossible to eliminate this psychic effect, but it can readily

be measured when an inert substance is given as a control. It is implicit that this placebo control should be prepared and administered in a form indistinguishable from that of the test drug. Medications should be administered in such a manner as to equate the effects of prior medication and of time; this can usually be best accomplished by resorting to some planned randomized order of administration. When it is wished to compare the effectiveness of the test drug with a known effective analgesic, it is advisable also to include this analgesic as a standard along with the placebo control and test drug as part of the experimental design.

*The patient.* In any sampling of patient populations we inevitably encounter a certain number who fail to discriminate between known effective and ineffective drugs. These persons may be those who fail to react to any medication or who are equally responsive to all. The latter are usually designated as the "placebo reactors" and it has been the common experience of investigators who employ placebo controls that from 30 per cent to over 50 per cent of their patients react positively to placebo (8, 9). This has also been the experience of the authors, although we have been impressed, as have Beecher and his associates (10), with the fact that most patients react inconsistently to placebos and it is a misnomer to call these people "placebo reactors." Although it may at first glance appear desirable to eliminate from a clinical study those patients who react to placebos, it is of some interest that Beecher and his associates found that the consistent non-reactors as a group did not present a "normal" picture on the basis of psychologic studies, this group being far more rigid and emotionally controlled than the average for their age and background (10). The inclusion of both a known effective standard and a placebo in the experimental design affords some indication of the ability of the group as a whole to discriminate, and thus serves as a measure of the sensitivity of the study. It follows, therefore, that any absolute measure of analgesia, either in terms of the number of patients responding or any other arbitrary scale, will depend upon the proportion of discriminators to non-discriminators in the group, and must be interpreted in this light when comparing drug effects in different groups of patients. The ideal design is to have each patient receive all the medications in the study, and thus serve as his own control. When this is not possible, one must not only attempt to equate patients in each group as to age, sex, disease and other pertinent characteristics, but one must use groups that are sufficiently large to minimize the effects of these unpredictable group differences.

*The setting.* There is good reason to believe that the setting in which drugs are administered is intimately related to the patient's responses. The symbolism of the pain, and even of the ritual of receiving medication, is undoubtedly different to a patient who knows or suspects that he has an incurable or fatal disease than it is to a patient who is recovering from a curative operative procedure. In cases in which alteration of the reaction to pain may be the primary effect of the analgesic drug, this difference will be no small factor in determining the relative effectiveness of the medication. The possibility also remains that certain analgesics may be more effective against some painful states than against others, even without specific curative effects.

*The observer* in any of these studies is an inseparable part of the setting.

Any prejudices that the observer brings to the situation will be reflected in the responses that he elicits from the patient. For this reason it is essential that the observer be uninformed as to the identity of the drugs used in any analgesic study. If more than one observer is to be employed, it is equally important that each be treated as still another variable and the study designed accordingly.

*The investigator's concepts.* Analgesia is by definition the loss of sensibility to pain but the criteria used to determine this condition will depend on the operative concepts of the investigator. The definition of adequate analgesia or a "positive response" to the medication will depend largely upon his clinical material and his goals. Investigators (including the authors) who are concerned with the problems of patients suffering from chronic or unremitting pain may be inclined to measure the effect of the drug not only in terms of its intensity but also in terms of its magnitude over time. Others concerned with more acute pain may find it more feasible to place the emphasis on the estimation of peak effects. Which of these is the superior measure of drug action? Each investigator would probably argue for his own particular method, and each method would have merit, depending upon the circumstances under which the drugs are tested. This is but another reason why we may expect different estimates of the relative potencies of drugs; yet each estimate may be valid in its respective situation. We cannot conceive of a single yardstick that would be valid or feasible under such a wide variety of clinical circumstances. Even within a single framework there are many ways of defining analgesia. It may be in terms of quantal measures such as: "at least 50 per cent relief of pain," "complete relief of pain," "presence or absence of comfort," and "good" versus "bad" days; or in terms of quasi-quantitative measures such as: changes in "pain intensity," degree of "pain relief," and estimates of "therapeutic effectiveness" based upon a compromise between degree of pain relief and incidence and severity of side effects. It would thus appear that each investigator is speaking his own language and we voice the hope that, in order to avoid the pitfalls of a Tower of Babel, clinical investigators may eventually come together on some common ground regarding a more uniform criterion of analgesia. Whichever criteria are used we must recognize that in our present state of incomplete knowledge of pain mechanisms, all such measures are arbitrary. Regardless of the criterion adopted, all measurements in the clinical situation are subject to uncontrolled variables, and in each study there will be errors of random sampling. These chance variations may be reduced by increasing the size of the series, and by proper design of the experiment. They can never be eliminated entirely. It is always advisable, therefore, to design studies so that the data may be subjected to statistical tests for estimation of the significance of the results.

In discussions of this sort there always seems to arise a consideration of the properties of the hoped-for "ideal analgesic." We all agree that we are looking for a drug that is potent, that will be effective by all routes of administration, that is predictable in its action, and that has no side-effects or toxic effects of any sort. However, careful scrutiny of the problem leaves us with the impression that the variety of effects required in the individual clinical management of pain

is greater than can possibly be achieved in any single drug. If one is concerned with patients who have chronic and unremitting pain due to incurable cancer (as we are), the ideal analgesic would have a very prolonged action, possibly some sedative properties, and the power to induce a considerable degree of euphoria. If, however, one is prescribing for the obstetrical patient, the usual postoperative patient, or the patient about to undergo a minor surgical procedure, such an analgesic would be considered far from ideal.

In searching for new analgesics we are each working in our own little niche, hoping to uncover agents that will best satisfy our purposes in ministering to the needs of our patients. This is as it should be. Perhaps we cannot expect that all needs will be satisfied by a single drug or that the problem can be adequately approached by any single analgesic testing method.

## SUMMARY

The goal of the investigator concerned with the evaluation of an analgesic agent is to be able to predict its clinical usefulness from his necessarily limited research findings. His ability to predict will be influenced by a number of factors in the clinical situation, not the least of which is the symbolic significance that man has since earliest times attached to pain and the agents employed to relieve it. The major variables concerned in the evaluation of analgesics are those associated with the drug, the patient, the setting, the observer and the investigator's own operational concepts. How these may influence the results of clinical studies is discussed. Because of the diversity of the clinical problems, there can be little hope at the present time for uniformity in the approach to evaluation of analgesics.

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# THE EVALUATION OF SEDATIVES*

ROBERT C. BATTERMAN, M.D.

*The Department of Medicine, New York Medical College, Bird S. Coler Memorial Hospital and Home for the Aged, Flower and Fifth Avenue, and Metropolitan Hospitals, New York, N. Y.*

The number of new agents introduced recently as sedatives has focused the attention of the chemist, pharmacologist and clinician upon the problem of their evaluation. Sedative drugs are defined as agents which depress the central nervous system. This definition, however, is too broad. The multiplicity of functions of the central nervous system allows classification of many drugs with respect to specific depression of a particular center or brain area. We thus have "cough" sedatives, which depress the cough center; anticonvulsants, which depress the cortical areas related to motor activity; and analgesics, which exert part of their effects by way of a sensory blockade of the lower subcortical areas. Excluding these specific groups of medications, there remains a large group of drugs which have been lumped into the general classification of sedatives per se. Dependent upon the degree of central nervous system depression, there exist many different degrees of true sedation. Anesthesia with loss of consciousness and depression of all cortical activity represents the maximum degree, or state of narcosis. Hypnotics or soporific agents result in sleep but cause no alteration in motor activity and sensory perception. Somnifacients are somewhat less potent than hypnotics and may result in drowsiness without sleep. The mildest effect is that of quieting or counteracting overstimulation of the central nervous system, so that the patient regains normalcy without becoming drowsy or sleepy. The term "sedative" should be reserved for this type of effect. It is a general rule that for each class of "sedative" drugs there exists an optimum effect which cannot be surpassed unless toxic doses are administered. Thus, mild sedative drugs cannot have a satisfactory hypnotic effect, nor can hypnotic drugs induce anesthesia. On the other hand, the more potent drugs which induce anesthesia can provide any lesser degree of "sedation" by means of progressively lower doses. A definite gradient of effect exists, which should be considered in selection of a particular sedative. This gradient may be related to locus of action as well as to potency and dose of the drug. If the major site of action is upon the cerebral cortex, as in the case of bromides and phenobarbital, such drugs will have their greatest usefulness in patients manifesting primary or reflex hyperexcitability of this portion of the brain, for example, in febrile states, delirium, hyperthyroidism or epileptiform conditions. Since depression of other portions of the central nervous system may not be evident unless toxic doses are administered, bromides and phenobarbital are not satisfactory hypnotics unless the insomnia is related to a hyperirritable cerebral cortex.

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With any single type of drug depressing the central nervous system there may be an overlapping of effects because of lack of specificity. Thus, analgesic agents such as morphine and meperidine may result in sedation as well as relief of pain. On the other hand, sedative drugs do not alleviate pain per se, except in anesthetic doses. Certain barbiturates are hypnotics primarily, but in large doses are also anticonvulsants. Other barbiturates (principally phenobarbital) which have their greatest pharmacologic action upon the cerebral cortical areas, are more effective as anticonvulsants than as hypnotics.

Until recently, the evaluation of the depressants of the central nervous system which are classified as true "sedative" drugs was dependent upon whether or not their optimum effects manifested themselves by means of controlling symptoms in the patient's anxiety state or "nervousness." With the advent of the alkaloids of Rauwolfia serpentina and the newer synthetic compounds such as chlorpromazine and Miltown,® a new type of sedative action has been revealed. It goes beyond the usual expected effect of relaxation, relief of tension, control of nervousness and irritability. The usefulness of these preparations for the treatment of frank or borderline psychoses, which heretofore were not responsive to the usual "sedative" drugs, requires an additional classification. The term "tranquillizer" has been used, although exactly what it means with respect to pharmacologic action is not clear. There is no question, however, that these agents possess unusual properties that transcend their employment as sedative drugs per se. Since the evaluation of these properties is in the realm of psychiatry, they will not be considered in this discussion. These drugs, however, possess, in addition, mild sedative properties which are of value in a multitude of non-psychotic emotional disturbances. When used for this purpose, they should meet the requirements of sedative agents, regardless of overlapping pharmacologic actions.

## INSOMNIA

It is evident from the foregoing that an evaluation of sedative drugs is, first of all, dependent upon the particular requirement of clinical usage. Foremost is the use of sedative drugs as hypnotics. The treatment of insomnia, however, requires more than a simple administration of a drug. Since most problems related to insomnia involve environmental conditions, habit, or emotional disturbances, adjustment of these factors is of greater importance than drug therapy. Nevertheless, hypnotic agents are frequently prescribed and therefore their function should be clearly understood. There is no question that this function is the "induction of sleep" within a reasonable period of time, gradually, and with transition into natural sleep.

An analysis of the sleep pattern of a normal person is instructive. The subject, after a latent period of varying length, dependent upon habit, sleeping conditions and perhaps mental processes, will lapse into a condition of progressively deeper unreceptiveness. The depth of sleep follows a characteristic curve, and reaches its peak approximately at the end of the first hour; it then decreases, at first rapidly and then slowly, until awakening occurs. The duration of sleep

varies from person to person and among age groups, but the average for the usual adult population is approximately eight hours.

An analysis of the sleep problems noted in patients with insomnia discloses 4 major disturbances: 1) Delay in the onset of sleep, with a normal pattern once sleep is initiated. 2) Short duration of sleep, with delayed or normal onset. 3) Fitful, disturbed sleep, with multiple crests and troughs for the entire duration. This pattern may be superimposed upon any of the two previous ones, so that several varieties are possible, but it is usually associated with a disturbing symptom complex such as pain, pruritus, cough, dyspnea, or the distress of congestive heart failure, whereas the insomnia of the first two categories is more often related to emotional or psychic problems, habit, and environmental abnormalities. 4) The fourth disturbance in sleep pattern is a complete reversal, with the patient awake at night and asleep during the day. Such patients, during the period of sleep may, in addition, manifest any of the combinations of the three varieties discussed previously. Reversal of the sleep pattern is usually seen in patients with advanced cerebral arteriosclerosis or disease of hypothalamic areas, but occasionally in patients who have been under the influence of large doses of narcotics or sedative drugs.

In evaluating hypnotic drugs, it is necessary to survey the types of patient utilized as subjects. Patients with a reversed sleep pattern are not suitable subjects for such studies. Since the responsiveness of a patient to a hypnotic agent is dependent upon the natural receptiveness of the individual to initiation of the sleep rhythm, the effectiveness of hypnotic agents, regardless of potency, is relatively nil if they are administered during the early wakeful hours. Thus, for the normal individual a hypnotic agent will not be effective if administered in the morning but might be very effective if administered during the evening hours. The same rule applies to the patients with reversed sleep rhythms. The administration of hypnotics to these patients at night would serve no useful purpose. The increased dose necessary to be effective results in a mentally confused patient, for the patient is depressed day and night. Hypnotic agents should be studied, therefore, with regard to the four major disturbances of sleep patterns. Patients with a prolonged latent period might be benefited by an ultra short-acting preparation since, once sleep is initiated, there will be a normal pattern. Patients with a short duration of sleep might be useful subjects for long-acting medications. Patients with fitful sleep, secondary to concurrent disturbing symptoms, might be fit subjects for the evaluation of combinations of sedatives and other medications for control of specific symptoms.

As part of the determination of effectiveness, the study should attempt to duplicate the possible use of hypnotics for both hospitalized patients and those who are ambulatory at home. A preparation might be highly effective for occasional use at home but might be relatively ineffectual for hospitalized patients. The routine of hospital living, the association with other patients, and the nursing facilities, to mention only the most important factors in the environment of such patients, might negate any results anticipated from less demanding conditions.

## CRITERIA FOR HYPNOTIC AGENTS

The first criterion which a hypnotic agent should meet is the ability to induce sleep in a reasonable period of time; such sleep should occur gradually and merge into natural sleep. This should be accomplished with a usual therapeutic dose and the effect should be predictable in a high percentage of cases whenever a specific type of sleep disturbance is treated. Additional criteria, enumerated to satisfy demands for an ideal hypnotic, are that the induced sleep should not be preceded or replaced by cerebral stimulation nor followed by a hangover or other manifestations of prolonged effect. Therapeutic doses should not influence the cardiovascular, pulmonary or respiratory system or depress any vital function of the body. Undue care should not be required, because of the diseased state, the patient's age or the functional capacity of the liver or kidney, which factors might play a role in the excretion, metabolism or dissipation of the drug. Idiosyncrasies unrelated to pharmacologic effect of the drug should not appear following its administration, or at least should be rare. The medication should be easily administered and, regardless of the route, should be well tolerated. Tolerance and cumulation should be minimal, and habit and addiction liability should be small or preferably nonexistent.

Among the many hypnotic agents available today it is clear that none satisfies these ideal criteria. Barbiturates, which are the most popular at present, satisfy the criteria the least. It is no wonder, therefore, that many new chemical entities are under intensive investigation to delineate their usefulness as sedative agents. The field is wide open, since no specific chemical structure is associated with sedative effect. Sedation is possible with a host of heterologous compounds ranging from simple inorganic salts, such as bromides or magnesium compounds, to complex structures such as propanediol, glutarimide, carbamide or various antihistaminic derivatives. A discussion of each group would not be within the scope of this paper. It must be emphasized that, with few exceptions, these nonbarbiturate sedative agents are new, and much more investigative work has to be carried out before their ultimate effectiveness and safety are known.

The possession of hypnotic properties by a drug does not mean that it also possesses satisfactory sedative properties for daytime use in the control of symptoms in the over-anxious or nervous patient. This problem should be solved in its own right, with due allowance for the following principles. Effectiveness represents satisfactory control of the unpleasant symptoms labeled "the anxiety state," but without daytime drowsiness, impairment of mental processes, sluggishness of movement, or loss of libido. As in the case of analgesic agents, this satisfactory response should be evaluated in both ambulatory and hospitalized patients, when the medication is administered either as a single dose, an occasional dose for a short period of time, or in prolonged dosage (two to four times daily) for weeks or months. The effectiveness, likelihood of untoward reactions, and possible cumulative toxicity vary for each type of study and each group of patients. The advocates of the newer sedative agents have neglected this breakdown in terms of clinical usage. Results are based on "lumped" statistics

and may not reflect the general practitioner's experience with the same drug under different conditions. The criteria for an ideal daytime sedative parallel those for hypnotic agents. The usual therapeutic dose should result in satisfactory control of a specific anxiety state with a high predictability and little likelihood of untoward reactions. All other criteria are the same for both types of medication. The newer agents have not been used sufficiently to determine accurately to what degree they meet these criteria.

Methods of studying effectiveness are subject to considerable dispute. Conditions prevailing during studies are so variable that it is unusual for two investigators to agree on the results. The predominantly subjective aspects of the symptom complex and the response to therapy have led many observers to limit the variables, and to rely upon "double blind" techniques or other artificial experimental tools. It is our contention that such procedures do not reflect the use of these medications in the usual manner prescribed by physicians. Studies to determine the effectiveness of sedative or hypnotic drugs should be conducted with these factors in mind. Unless the results can be duplicated by the general practitioner, the data are of interest only so far as the particular study group is concerned. The conclusions should not be carried over to the entire field of sedation.

## CONCLUSIONS

Drugs, no matter how closely they satisfy the ideal criteria for sedation, are not the solution for the problem. Since an emotional disturbance which requires psychic adjustment is the predominant etiologic factor in most patients, the continuous use of sedatives is not good medical practice. Sedative agents have several advantages when used for temporary control of the patient's complaints, but abuse may lead to habituation and cumulative toxicity. Knowledge of their shortcomings allows their beneficial effects to be properly used.

# ADDICTION LIABILITY OF ANALGESICS: TESTS AND RESULTS*

NATHAN B. EDDY, M.D.

*National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Maryland*

It is generally recognized that tolerance and dependence develop when the administration of morphine is prolonged. Most everyone realizes that there is a similar risk with substances derived from morphine, although the degree of risk may vary from one derivative to another. It is a fact, too frequently overlooked, that a similar liability is associated with the new synthetic analgesic substances, of which there are now many types. For this reason, it is pertinent to discuss the sources of information regarding the addiction liability of these new substances and the conclusions which may be drawn from these data.

Addiction to opiates, to morphine and its derivatives, and to the synthetic analgesics with morphine-like effect, has three components—tolerance, physical dependence and psychic dependence. The unmistakable proof of addiction to these substances lies in the demonstration of physical dependence, and physical dependence is demonstrated by the development of a typical abstinence syndrome. According to Federal law, any drug is an opiate which is found to have an addiction-forming or addiction-sustaining property similar to that of morphine, and the law provides the means to bring opiates, whatever their chemical nature, under Federal narcotics control. Control is not intended to hamper scientific development or medical practice. It is for the good of all, and specifically for the prevention, so far as possible, of the abuse of addicting substances. Everyone should recognize the worthiness of these objectives and, moreover, should realize that when it is deemed necessary to bring a substance under narcotics control, one should be duly watchful and properly conservative in the use of such a drug, just as one would be with drugs of the same type with which one is already familiar.

There are, so far, only two sources of information on addiction-forming and addiction-sustaining liability: 1) gradual accumulation of clinical experience after a new agent has been introduced, informing of danger and damage only after the damage has been done, and 2) the work of the National Institute of Mental Health Addiction Research Center at the Public Health Service Hospital at Lexington, Kentucky (1). In the latter institution all observations are made on addicts and post-addicts and the question has arisen repeatedly of the validity of predictions based on conclusions from studies on such a population. There are two major justifications for the work and the conclusions drawn. It has already been said that a reason for narcotics control is avoidance of abuse. In this connection, observations indicating the attractiveness of a new drug for the addict population are pertinent for prediction as to the likelihood of abuse. Also the

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predictions made to date from the work at Lexington, especially with regard to the more dangerous drugs, have been borne out in clinical practice.

### ADDICTION TESTS

Given a new drug, of which the addiction liability is to be assessed, four types of experiment are carried out at Lexington. First, starting with small doses based upon previous experience with the drug, the agent is administered to post-addicts by various routes, observations are made for the appearance of morphine-like or other effects, and note is made of the individual's subjective reactions. The drug is given as an unknown, but the patient is allowed to express himself as to what drug he may have received in comparison with his previous drug experience. He may say, for example, that it is like heroin or morphine or some other drug which he has taken previously.

Second, individuals who have been receiving morphine regularly, and who have been stabilized on a daily dosage of 200 to 300 mg., are abruptly deprived of the drug and at the thirtieth hour of abstinence receive a dose of the experimental substance. The size of this dose is based upon the experience in the first experiment as to its morphine-like effect. Prior to its administration, the abstinence syndrome is evaluated according to the Himmelsbach scale (2) and this evaluation is continued hour by hour in order to determine to what extent the new agent is able to suppress the morphine abstinence phenomena. The suppression, if any, is compared in degree and duration to that obtained with a 30-mg. dose of morphine (Fig. 1).

The third experiment consists of the substitution of the new drug for morphine in an individual stabilized on morphine, as indicated in the second experiment. An attempt is made to substitute the new drug at such dosage levels and intervals of administration as to maintain the individual's addiction, that is, to prevent completely the appearance of abstinence phenomena which would otherwise occur because of the cessation of morphine administration (Fig. 2).

It should be pointed out that no substance has been found which will significantly suppress morphine-abstinence phenomena, or which will be a substitute for morphine in an established addiction, maintaining the addiction and preventing the appearance of abstinence symptoms, which will not itself cause addiction. Furthermore, possessing the power to sustain, as well as to form an addiction, defines a substance as an opiate.

The fourth experiment is carried out with agents most likely to come into clinical use, or with new chemical types. To individuals previously addicted to morphine or related drugs and free of drugs for some months, the new agent is administered regularly around the clock, at an interval and dosage based upon the experience gained to this point. The dose is increased as rapidly as possible, if cumulative effect or toxic reactions do not occur. Administration is continued usually for about thirty days. At that time administration is stopped abruptly and the individual is followed for the appearance of abstinence phenomena, which again are evaluated according to the Himmelsbach scale and compared with the abstinence phenomena which occur after a similar period of administration of morphine.

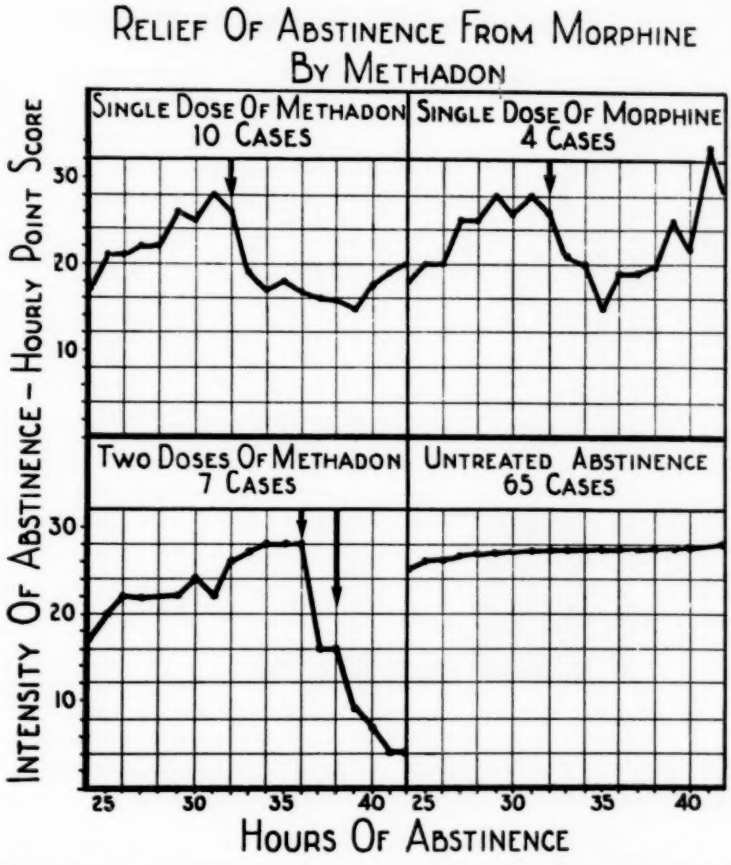


FIG. 1. *Upper left.* Average point score of 10 subjects who received 21 mg. of methadon at the 32nd hour of abstinence. *Upper right.* Average point score of 4 of the same subjects who received 30 mg. of morphine at the 32nd hour of a subsequent abstinence. (Reproduced with permission, from Isbell and Vogel (3).)

From these observations one gets a qualitative and, to some extent, a quantitative comparison of the new agent with morphine in terms of its ability to produce morphine-like effects, to induce the subjective reactions which the addict values, to suppress morphine abstinence phenomena or sustain morphine addiction, and to cause physical dependence and tolerance in a former morphine addict.

Not long ago an experiment was started at the University of Michigan to attempt to assess addiction liability, using the monkey as the experimental subject. This experiment is continuing and at the moment gives promise of the development of a technique for evaluation of addiction liability which may be of value as a basis for prediction of what may occur in man. The results to date are in most respects parallel to the experience previously obtained at the Addiction Research Center in Lexington. The experiment is now being subjected to a critical appraisal for the assessment of addiction phenomena by the administration to monkeys of a fairly large group of substances as unknowns, and comparing the results with that of administration of morphine simultaneously in another

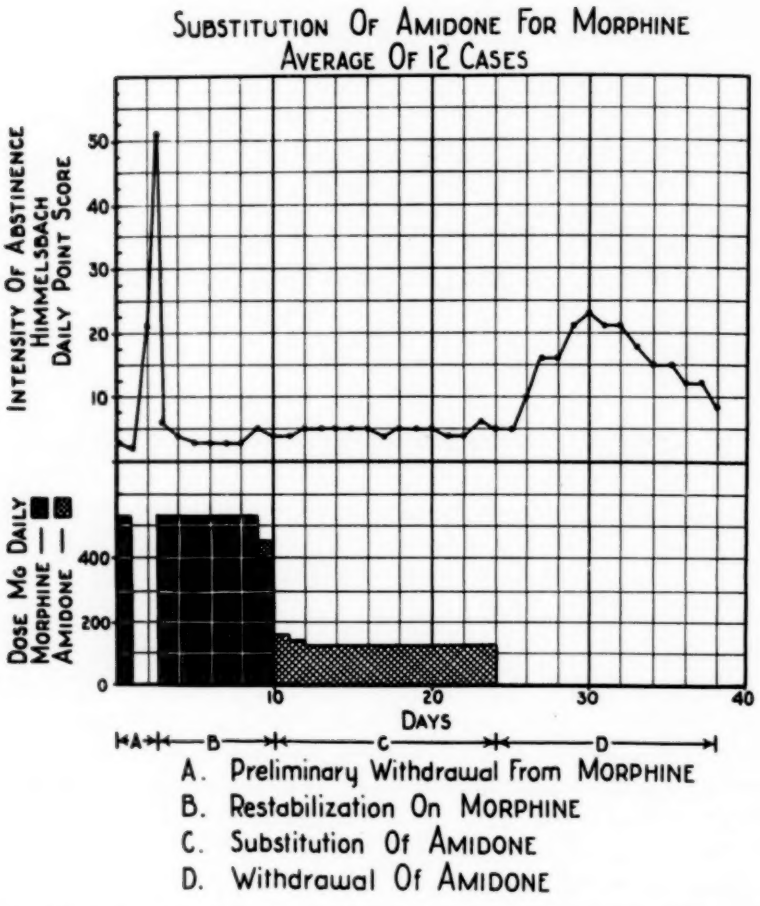


FIG. 2. Amidone = methadon. (Reproduced with permission, from Isbell *et al.* (4).)

group of these animals. As in the Lexington experiments, the drugs are administered around the clock, at an interval and dosage based upon the duration and intensity of action. Probably no prediction of addiction liability will be entirely valid without observations on man, but if the monkey experiments are successful, they may save a great deal of time and perhaps greatly facilitate the subsequent tests in man.

The work at Lexington has been going on for some twenty years. It was begun by Dr. C. K. Himmelsbach and is continuing under the direction of Dr. Harris Isbell. Many agents have been administered, such as morphine derivatives and different types of synthetic substances, some of which have come into clinical use. Tables 1 and 2 summarize some of the Lexington results.

### ADDICTION VS. ANALGESIA

Early in this work Dr. Himmelsbach (9) questioned whether a comparison of only the physical-dependence effects of two drugs would give a true picture of the relationship of their respective addiction liabilities. He expressed the opinion

TABLE 1

| | Analgesic Effect | | | | Substitution in Stabilized Morphine Addicts | | References |
|---|---|---|---|---|---|---|---|
| | In mice | | In man | | | | |
| | $ED_{50}$* (mg./Kg.) | Duration (min.) | Dose (mg.) | Duration (hrs.) | Individual dose (mg.) | Doses/24 hrs. | |
| Morphine | 2.1 | 129 | 10 | 4–6 | 50–75 | 4 | (2, 5) |
| Codeine | 14.2 | 67 | 60 | 4–6 | 259 | 4 | (6) |
| Dihydromorphine | 1.8 | 154 | 5–10 | 4–6 | 15 | 4 | (7) |
| Diacetylmorphine (Heroin) | 0.9 | 69 | 2–5 | 2–4 | 71 | 4 | (8) |
| Dihydromorphinone (Dilaudid) | 0.34 | 133 | 2–4 | 3–4 | 7–12.5 | 4–6 | (9) |
| Methyldihydromorphinone (Metopon) | 0.47 | 119 | 4 | 3–4 | 7 | 8 | (10, 11) |
| Dihydrocodeinone (Hycodan) | 3.2 | 85 | 10–20 | 3–4 | 60 | 4 | (12) |
| Dihydrohydroxymorphinone (Numorphan) | 0.17 | 122 | 1–2 | 4–5 | 6 | 8 | (13) |
| Dihydrohydroxycodeinone (Eucodal) | 1.4 | 89 | 5–10 | 4–5 | 75 | 4 | (7) |
| Dihydrodesoxymorphine-D (Desomorphine) | 0.18 | 58 | 1 | 1–3 | 10 | 6–8 | (10) |
| 6-Methyldihydromorphine | 4.0 | 140 | 31 | 4–6 | 83 | 3–4 | (14) |

* $ED_{50}$ = Dose producing significant analgesia in 50 per cent of animals; derived from probit analysis of the data.

that comparisons of the relationships of both the physical-dependence and analgesic actions of narcotic drugs would be significant and that plotting the intensity and duration of these two facets of a drug's action could give an indication of the likelihood of the development of addiction in clinical practice. If in Figure 3 curve A represents the analgesic and curve B the physical-dependence or addictive action of a hypothetical compound, the repeated administration of such a drug only for the relief of pain should be much safer from the standpoint of addiction than if these relationships are reversed. The hypothesis assumes that analgesic and physical-dependence actions are independent properties of the drug, and that in the hypothetical case the latter has waned completely before it is necessary to repeat administration of the drug for relief of pain. It is known that the optimal condition for development of physical dependence is repeated administration at a regular interval, an interval not necessarily related to the duration of analgesic action. These relative durations of analgesic and physical-dependence effects are significant for clinical practice, but not so much so with respect to the risk of addiction. For the latter, intensity of morphine-like effect is the more important, though prolongation of action may make a drug more attractive, and extreme shortness of action, even of intense action, may make it less attractive and less liable to abuse because of the cost and difficulty of very frequent injections.

In line with the argument just presented, data on analgesic action as well as on addiction liability are shown in Tables 1 and 2. Data from our laboratory

TABLE 2

| | Analgesic effect | | | | Morphine-like effects in post-addicts | | | Direct addiction | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | In mice | | In man | | | | | | | |
| | $ED_{50}$* (mg./Kg.) | Duration (min.) | Dose (mg.) | Duration (hrs.) | Single dose† (mg.) | Suppress. of abstin sympts. (mg.) | Substitution dose (mg.) | Individual dose (mg.) | Doses/24 hrs‡ | |
| Morphine | 2.1 | 129 | 10 | 4–6 | 30 | 30 | | 30–60 | 4 | (5, 10, 15) |
| *l*-Methadone | 0.8 | 80 | 5 | 4–6 | 15 | 11 | 15 | | 4 | (5, 16, 17) |
| *d*-Methadone | 26.0 | 74 | — | | None [90] | None | None | | | (16, 17) |
| *l*-Isomethadone | 1.2 | 75 | 10 | 4–6 | 15 | 30–45 | 90 | 20–90 | 4 | (5, 16) |
| 4,4-Diphenyl-6-dimethylamino-3-hexanone | 3.7 | 54 | 10–20 | 4–5 | 45–60 | 60–90 | 60 | | 4 | (18) |
| Meperidine (Demerol) | 10.0 | 109 | 50–100 | 3–5 | 75–100 | 75 | 60–120 | 75–100 | 4 or 8 | (19) |
| | | | | | | | | 100–210 | 6–12 | (20) |
| Ketobemidone (Cliradon) | 1.6 | 127 | 5–10 | 2–3 | 30 | 30 | | 30–80 | 4–5 | (21) |
| Alphaprodine (Nisentil) | 1.9 | 88 | 10 | 2–3 | 100 | 60–90 | | | | (21) |
| Levorphan (Dromoran) | 0.5 | 120 | 1–2 | 4–6 | 3.5 | 5–7 | | | | (22) |
| Dextrorphan | None | | None | | None [100] | None | None | | | (22) |
| Levomethorphan | 3.0 | 136 | — | | 20 | 22 | 40–50 | | 3 | (23) |
| Dextromethorphan (Romilar) | None | | None | | None [100] | None | None | | | (23) |
| 3-Ethylmethylamino-1,1-di-(2′-thienyl)-but-1-ene | 2.4 | 97 | 50 | 4–6 | 60 | 60 | 60 | 15–60 | 8 | (24, 25) |
| Ethyl-1-methyl-4-phenyl-hexamethyl-eneiminecarboxylate | 42.0 | 94 | 50–100 | 3–4 | None [200] | None | None | | | (26, 27, 28) |

* See footnote to Table 1.

† Figure in brackets after "None" denotes largest dose tried.

‡ Doses/24 hours for substitution if direct addiction experiment was not tried, as indicated by blank in individual dose column.

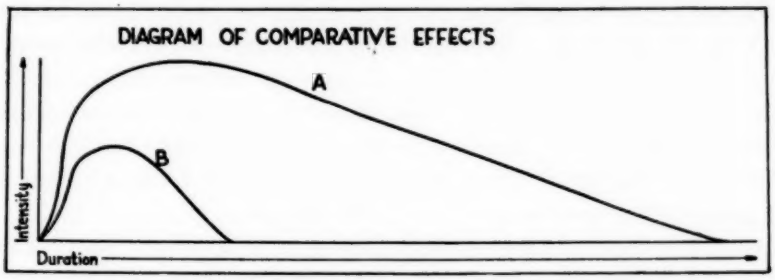


FIG. 3. *See* text for explanation. (Reproduced with permission from Himmelsbach (10).)

are included in those on analgesic action, because the experimental method permits fairly precise expression of duration of action. The analgesic doses for man are based so far as possible on the work of Dr. Beecher and his associates on postoperative pain and represent equivalence to the analgesic effect of a 10-mg. dose of morphine. All of the compounds listed in Table 1 have morphine-like effects and in adequate amount will suppress morphine-abstinence phenomena; consequently only the amounts and intervals of administration for smooth substitution in stabilized morphine addicts are shown.

Of the morphine derivatives listed in Table 1, in the terms of Dr. Himmelsbach's concept, heroin is particularly dangerous because the intensity of action has been stepped up and the duration of analgesia, but not that of the physical-dependence action, shortened. A similar situation holds for dihydromorphinone. Desomorphine is very intense but very brief in its effects and consequently not likely to be attractive or subject to abuse. 6-Methyldihydromorphine, methyldihydromorphinone and dihydrohydroxymorphinone have the most favorable relationships between durations of analgesic and physical-dependence actions. In each case, duration of the latter seems to have been shortened without material decrease in the duration of analgesia. The intensity of action of dihydrohydroxymorphinone is so greatly increased as possibly to offset the small decrease in duration of the dependence action. Medical addiction has been described for each morphine derivative in this table (including desomorphine) that has come into clinical use.

In Table 2 are shown the doses of the synthetic substances which have morphine-like effects in post-addicts and which suppress the morphine-abstinence syndrome; the substitution doses are also shown. It is apparent that the suppressive dose is usually adequate as a substitute for morphine on continued administration. A direct addiction experiment was not carried out in all cases.

It is noteworthy that dextrorphan and dextromethorphan, which have no analgesic effect, and dextromethadone, which has very little such action (none in man), have shown no morphine-like effect or evidence of addiction liability. On the other hand, potent analgesics of four different chemical types, *viz.*, methadone, meperidine, morphinan and dithienylbutenylamine derivatives, have shown positive results in each type of experiment designed to test addiction liability.

It is difficult to give the whole picture by figures alone. Methadone, for example, in a single dose has a prolonged morphine-like effect which many addicts find attractive. It substitutes for morphine very smoothly in an established addiction. On abrupt withdrawal of methadone, the abstinence syndrome develops slowly, is prolonged and is not, as a rule, intense. Substitution with methadone and its subsequent rapid withdrawal are the best means known to ease the withdrawal sickness of addiction to other narcotics.

Ketobemidone leads to demonstrable physical dependence very rapidly, and the interval of administration soon must be shortened in order to avoid preinjection abstinence symptoms. Its abrupt withdrawal is followed by an abstinence syndrome, very rapid in onset, very intense, and of short duration. These characteristics have been commented on in accounts of its clinical use (29, 30), and for this reason the abandonment of ketobemidone as a medicament has been recommended.

The figures for levomethorphan are particularly unfavorable, since this drug need only be given three times a day to substitute adequately for morphine yet its analgesic action, compared to that of morphine, does not last appreciably longer. It has not come into clinical use and these figures suggest that it should not.

Dr. Himmelsbach carried out two experiments on direct addiction to meperidine (19, 20). In one, the subjects were allowed to increase the dose and decrease the interval of administration as they wished, and very large amounts were taken. In the second experiment, the usual clinical doses were given at the usual intervals without change for periods up to two months. Even in these cases a definite abstinence syndrome appeared after abrupt withdrawal. When morphine was administered four times a day in a dose of 10 mg. without change for thirty days, there was also a definite abstinence syndrome on withdrawal. The Himmelsbach point score was slightly greater for morphine than for meperidine when the doses were kept at therapeutic levels.

The discovery of meperidine less than twenty years ago was of tremendous importance, but unfortunately when it was introduced into medicine the impression was fostered that it was relatively non-addicting, as was the case years before with heroin and dilaudid. Such popular impressions, contrary to fact, inevitably result in addiction in many individuals who should have been spared that experience. The impression of the low addiction liability of meperidine seems to persist although, as the use of the drug has increased, cases of addiction to it have increased. Rasor and Crecraft (31) reported 457 meperidine addicts admitted to the Lexington Hospital in the thirty months ending September 30, 1953, nearly half of them members of the medical and allied professions.

Synthetic analgesics like the natural alkaloids, morphine and its derivatives, vary in the intensity of their addiction liability and neither group should be considered more dangerous than the other in this respect. Each new analgesic, regardless of origin, if it is to be used in medicine, must be assessed for addiction liability, and the result balanced against the possible therapeutic advantages. If the drug is shown to be addicting, as has been the case for every potent anal-

gesic so far, and yet has advantages warranting its clinical use, for our own protection we should employ the same precautions with the new drug as we would with morphine.

Finally, hope should not yet be abandoned for the discovery of a good analgesic with low addiction liability. The hexamethyleneimines, shown recently to have some analgesic action (26, 28), may be an important step in this direction. Two of them have been tested at Lexington and both have shown no morphine-like effect and no ability to suppress morphine-abstinence phenomena. They are not very powerful analgesics clinically and it remains to be seen whether the absence of addiction liability is truly characteristic of the group as analgesic potency is increased.

## SUMMARY

A description is given of the tests in use at the National Institute of Mental Health Addiction Research Center for determination of the addiction liability of analgesics. The tests consist of administration of single doses for detection of morphine-like effect, attempt to suppress morphine-abstinence phenomena, substitution for morphine in an established addiction, and regular administration for a limited period. Data illustrative of the results obtained with morphine derivatives and with synthetic analgesics of different types are presented.

Synthetic analgesics, like the natural alkaloids, morphine and its derivatives, vary in the intensity of their addiction liability and one group should not be considered more dangerous than the other in this respect. A new analgesic substance, whatever its source, must be assessed for addiction liability and the result balanced against therapeutic advantages. If the result is positive, as with every potent analgesic so far tested, then for our own protection the same precautions should be exercised as with the use of morphine.

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# CLINICAL EFFECTIVENESS AND SAFETY OF A NEW SERIES OF ANALGESIC COMPOUNDS*

ARTHUR J. GROSSMAN, M.D., MAURICE GOLBEY, M.D., WILLIAM C. GITTINGER, M.D. AND ROBERT C. BATTERMAN, M.D.

*The Department of Medicine, New York Medical College, Bird S. Coler Memorial Hospital and Home for the Aged, Flower and Fifth Avenue, and Metropolitan Hospitals, New York, N. Y.*

Seifter *et al.* (1) reported on the analgesic properties of a new series of compounds of unusual interest. The chemical structure of these compounds, in contrast to the six-membered ring of meperidine (Demerol), is a seven-membered configuration. Since the results of animal work were suggestive of a high degree of analgesic potency without the secondary effects of morphine, it was thought advisable to evaluate these compounds in man as analgesic agents. The 3 derivatives (Fig. 1) included in the study,[1] were labelled Wy-401, Wy-682 and Wy-782, respectively, and for the sake of brevity will be referred to by these designations.

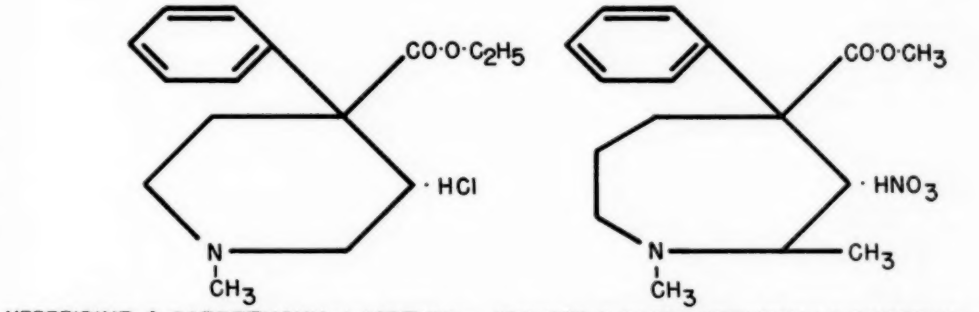


FIGURE 1

* Read before the Fifty-Sixth Annual Meeting of the American Therapeutic Society, Atlantic City, N. J., June 2–5, 1955.

[1] Wyeth Laboratories, Philadelphia, Pa., graciously supplied us with the medication and gave other aid in connection with this investigation

As indicated in a previous report (2), the clinical evaluation of analgesic agents requires the utilization of patients with many types of pain, of various etiologies. To obtain a complete picture of the effectiveness of any analgesic agent, it is essential for the medication to be used under conditions equivalent to those in clinical practice. Any attempt to limit the variables of an analgesic study to a single disease entity, type of pain, or period of drug administration and observation, under artificial nonclinical conditions, does not reflect the clinical usefulness or safety of the medication. Such investigations may indicate that a particular agent, under the conditions of study, is effective and safe, but these data should not be accepted as final in the assessment of clinical analgesia with all its implications and ramifications.

## METHOD

Evaluation of an analgesic agent should consider the effectiveness and safety of the initial and subsequent doses, and the frequency and duration of administration. Since the ambulatory status of the patient plays a significant role, this factor should also be considered. We therefore used these compounds under the conditions encountered by any physician when a potent analgesic agent is required. No attempt was made to select the subjects with regard to severity, duration, type and etiology of the pain. The patients varied in age from 13 to 92 years and their pain was due to the usual medical and surgical conditions such as musculo-skeletal diseases, neoplasms with metastasis, postoperative discomfort, or pleurisy. Most of the patients had received other analgesic agents previously, so their requirements for analgesics were well known.

In order to be classified as satisfactory, the analgesic response had to be either "complete" or "moderate." Complete response required the subjective obliteration of pain for a minimal period of three hours, without, or with minimal, untoward reactions. A response was considered to be "moderate" if there was complete relief of pain for less than this time, between two and three hours, partial relief at least three hours, or relief as described in the first category but with untoward reactions which did not permit complete comfort. Any response less than this, even though analgesia occurred, was considered unsatisfactory and of no significance.

In regard to oral administration, our first concern was to determine a dose which would be safe and well tolerated and yet achieve satisfactory analgesia. On the basis of animal experiments, which indicated that both Wy-401 and Wy-682, when administered orally, had approximately one fifth of their respective potencies when given by injection, Wy-401 was initially supplied in 250-mg. tablets, and Wy-682 in 50-mg. tablets.

## RESULTS

### *Oral administration (Table 1)*

It became apparent at the beginning of the study that the 250-mg. oral dosage was not satisfactory. Of 49 patients, 33 or 67.5 per cent experienced untoward reactions when the medication was taken every four hours. The high incidence

TABLE 1

*Effectiveness and Toxicity of Various Doses of 1-Methyl-4-carbethoxy-4-phenylhexamethylenimine (Wy-401) Administered Orally to Ambulatory Patients*

| Dose (mg. q.i.d) | No. of Subjects | Satisfactory Analgesia | | Toxicity | | | | Unsatisfactory Analgesia Because of Toxicity | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Incidence | | CNS Symptoms | GI Symptoms | | |
| | | No. | Per cent | No. | Per cent of total | Per cent | Per cent | No. | Per cent |
| 250 | 49 | 18 | 36.7 | 33 | 67.5 | 87.8 | 12.2 | 8 | 16.3 |
| 125 | 46 | 16 | 34.7 | 18 | 39.1 | 61.1 | 38.9 | 7 | 15.2 |
| 75 | 65 | 29 | 44.6 | 21 | 32.3 | 38.1 | 61.9 | 5 | 7.7 |
| 50 | 38 | 27 | 71.0 | 2 | 5.2 | — | — | 0 | 0.0 |

of toxicity overshadowed the likelihood of obtaining a satisfactory analgesic response. The toxic symptoms usually occurred after several days to three weeks of drug administration. Upon administration of four doses daily for from one to three weeks, 8 patients who initially had satisfactory analgesia had toxic reactions and the drug had to be discontinued. Cumulative toxicity appeared to be an individual phenomenon, since in 18 subjects the prolonged administration for several weeks was free of toxicity, and in 2 instances, 500 mg. every four hours for several weeks was also without toxicity. The toxic manifestations with the 250-mg. dosage took the form of central nervous system disturbances such as dizziness, visual symptoms, headache, syncope and nervousness. Gastrointestinal complaints were minimal with this dosage.

The study was continued on the basis of achieving a dose which upon prolonged administration would be safe and yet furnish satisfactory analgesia. When the 75-mg. dosage level was reached, the toxic manifestations were predominantly gastro-intestinal, *i.e.*, nausea with or without vomiting, and epigastric distress. Satisfactory analgesia was achieved only when the 50-mg. level was attained. With this dosage, there was adequate analgesia in the majority of patients and the incidence of untoward reactions was negligible. It is of interest that the 50-mg. dosage was one fifth of that predicted from animal experiments. Thus, in man at least, there was no confirmation of the report that there is poor absorption of the drug from the gastro-intestinal tract.

Studies with Wy-682 yielded similar results. The 50-mg. and 25-mg. oral dosages had effects equivalent to those of the 165-mg. and 75-mg. dosages of Wy-401, respectively. Even the 10-mg. dosage was perhaps too large, because the incidence of toxicity was still too high.

Since the data favored the 1-methyl derivative of Wy-401, this drug was administered in dosages of 50 to 150 mg. four times daily to another group of ambulatory patients with a wide variety of medical conditions other than musculo-skeletal. In 29 such patients the medication was effective, with satisfactory control of pain in 20, or approximately 68 per cent. Slight nausea occurred in 1 patient and anorexia was noted in 1 patient, so that the incidence of untoward reactions was approximately 7 per cent.

The effects of Wy-401 administered orally were studied in 14 hospitalized patients with pain due to cancer. The dosage was in multiples of 50 mg. (four times daily). Satisfactory relief of pain was achieved in 7 patients, or 50 per cent. All of these had severe pain and although in the early phases the pain was controlled with oral medication, it was finally necessary in all to resort to more potent injectable analgesics. In our experience, Wy-401 orally is more potent than codeine and considerably less toxic.

In 12 patients who experienced no toxicity during the prolonged administration of 75 mg. of Wy-401 every four hours, this dosage was continued in order to determine the liability to addiction. Six of the 12 subjects took the medication for a period of eleven to eighteen weeks. Its use was then stopped because the patients failed to appear in the clinic at the appointed time. None of these 6, nor the remaining 6 who completed six months of continuous use, presented any withdrawal phenomena.

*Parenteral administration*

Table 2 shows the results with Wy-401 or the 1-methyl derivative, given in doses of 90 to 240 mg. intramuscularly to 51 patients for 57 trials. In all, 348 doses were administered. The medication was given as a single dose to 5 subjects; in the majority, it was repeated as required. At the beginning of the study it was noted that the duration of analgesia was longer than that noted previously with morphine or meperidine, so that it was not necessary except in a few instances to repeat the medication in four hours. When repeated analgesia was required, most of these subjects received the medication at intervals of from five to seven hours upon demand—usually in 5 or more doses. The greatest number of doses administered was 37 over a period of sixteen days. With the exception of postoperative patients and an occasional one with short-lived pain, the majority of the subjects received the medication for two days or longer. Thus, the responsiveness and development of tolerance or untoward reactions to repeated doses was followed in 34 patients.

Regardless of dose, etiology or severity of pain, satisfactory analgesia was obtained in 36 of the 57 patients, an incidence of 63.1 per cent. Although it would

TABLE 2

*Effectiveness of Parenteral Administration of 1-Methyl-4-carbethoxy-4-phenylhexamethylenimine (Wy-401)*

| Dose (mg.) | No. of Trials | Satisfactory Analgesia | |
|---|---|---|---|
| | | No. | Per cent |
| 90 | 11 | 7 | 63.6 |
| 120 | 23 | 13 | 56.5 |
| 180 | 7 | 5 | 71.4 |
| 240 | 16 | 11 | 68.8 |
| | *57* | *36* | *63.1* |

appear from these preliminary data that the likelihood of satisfactory analgesia is the same at any dose level, our experience with different doses in the same patient would indicate that a 180-mg. or 240-mg. dose may be more effectual for the severer types of pain. This is true for patients with malignancy, particularly if bone metastasis is a predominant factor. Postoperative patients do well with any of the doses, but the complete absence of sedation with Wy-401 often misled the hospital resident physician to prefer meperidine or morphine. The striking finding with Wy-401 was the duration of analgesia, which in many patients lasted from five to seven hours.

Untoward reactions were not noted at any dose level, when the drug was given only occasionally. At the beginning of the study, 3 patients receiving the smallest (90 mg.) dose every four hours for two to four days presented what appeared to be cumulative toxicity. However, much larger doses for longer periods of time in subsequent patients failed to confirm this observation. It is now felt that the original lot of medication contained an impurity, which has been removed. With these 3 exceptions, Wy-401 administered parenterally to bedridden patients, either in single or repeated dosage, has not induced any untoward reactions.

Wy-401, when administered in a dose of 240 mg. to a heroin addict and to a meperidine addict, had no effect upon the withdrawal symptoms of heroin or meperidine and did not result in any untoward reactions. An additional post-addict received repeated large doses without effect or toxicity.

At this time our studies with the 1–2 dimethyl (Wy-682) and 1–5 dimethyl (Wy-782) derivatives do not lend themselves to complete analysis. Thirty-two trials each with both drugs indicate that the analgesic effectiveness may not be as great as that of Wy-401. However, the optimum dose for either derivative has not been established. No patient has shown any untoward reactions with either drug.

Regardless of the derivative or the route of administration, none of the patients presented any abnormality in the blood-forming elements or other manifestations of serious toxicity during prolonged administration. Sedation, suppression of cough, disturbances in bowel or urinary function, dryness of the mouth, alteration of the pulse rate, depression of respiration or other secondary effects were not noted. Detailed studies of the rate of oxygen consumption per minute revealed no significant change following the injection of 120 mg. of Wy-401. Pruritis of a transitory nature was noted in 2 instances when the drug was given orally; this subsided promptly when the drug was discontinued.

## DISCUSSION

In a recent review (2) it was pointed out "our greatest shortcoming is a complete lack of moderately potent drugs to be administered by the oral route that could be used with reliance for the treatment of the largest number of patients, *i.e.*, the ambulatory patient with moderately severe pain from any medical or surgical condition." Our preliminary studies with the Wy-401, 1-methyl derivative of these hexamethylenimine compounds, indicate that this gap in the armamentarium of drugs for the relief of pain no longer exists. Wy-401 orally

possesses potency within the range of codeine. Its relative freedom from untoward reactions on occasional or prolonged administration is indicative of its superiority over other analgesics of equivalent potency used at present for the relief of pain in the ambulant patient. The injectable derivatives are at present not satisfactory for clinical usage, since they are racemic mixtures, and the dextro and levo forms possess mutually antagonistic properties. This probably explains the inadequacy of response to high dosages, as the stimulating effects overshadow the analgesic response.

## SUMMARY

1. 1-Methyl-4-carbethoxy-4-phenyhexamethylenimine (Wy-401), when administered orally to ambulatory and hospitalized patients with pain secondary to a variety of medical and surgical conditions, has been found to be an effective and safe analgesic.

2. For ambulatory patients the dose best suited for the achievement of satisfactory analgesia is 50 mg., repeated if necessary every four hours. Hospitalized patients may require 100 or 150 mg., depending upon individual requirements and tolerance.

3. The undesirable pharmacologic effects observed with prolonged administration of morphine, codeine or meperidine have not been observed to date with this new series of compounds.

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# EXPERIMENTAL LABORATORY METHODS FOR STUDYING ANALGESICS*

HERBERT S. KUPPERMAN, PH.D., M.D.

*Department of Therapeutics, New York University-Bellevue Medical Center, New York, N. Y.*

The search continues vigorously and systematically for a more effective non-addictive, analgesic compound controlling pain with no, or only minimal side effects, such as hypnosis or sedation. The development of a synthetic analgesic compound in 1939 by German workers (1) gave impetus to such investigations in an attempt to formulate a more effective less habit-forming, relatively non-toxic analgesic preparation. Until the development of meperidine and the demonstration of its analgesic qualities, the main progress in the field of new pain-relieving compounds was the synthesis of various opium alkaloid derivatives. It was claimed that some of these derivatives were more active therapeutically than the parent compound. Meperidine was the first synthetic non-morphine derivative which was proved to possess analgesic activity. It soon became apparent, despite the early enthusiasm, that this compound, when used over a long period of time, would lead to addiction. The habituation is not of the same order of magnitude as that of morphine, but is severe enough to create interest in the development of drugs of comparable pain dispelling non-addictive properties.

## TECHNIQUES FOR TESTING ANALGESICS

In the search for analgesic preparations it became apparent that an accurate, simple technique would have to be developed for the screening of the large number of compounds made available by the chemist.

### *Requirements*

Four important requirements had to be met before the procedure could be considered adequate (2, 3).

1. The technique for pain induction must be simple, readily reproducible and the endpoint should be easily discernible for accurate recording.

2. The method should be of sufficient sensitivity to distinguish between graded doses of the analgesic agent to be tested. In other words, within the limitation of activity of the compound a dose-response relationship should be demonstrable between the amount of material administered and the response, or percentage of animals responding, to the testing procedure.

3. The technique should be capable of demonstrating analgesia induced by mild analgesic agents that are in clinical use at the present time.

4. The testing procedure should yield results which will provide a high predictability of response with respect to clinical evaluation. This fourth prerequisite

* Read before the Fifty-Sixth Annual Meeting of the American Therapeutic Society, Atlantic City, N. J., June 2-5, 1955.

is by far the most important, and it is the most difficult to achieve, due to species variations with respect to pain reaction and tolerance. Many times the results obtained in the experimental procedure do not correlate well with the clinical adequacy of the drug when subjected to clinical trial.

*Subjects*

The methods employed for analgesic testing may make use of either the human subject or the experimental animal. Each has obvious advantages and disadvantages. Evaluation of the drug directly in the human will indicate the clinical analgesic activity of the preparation. Unknown preparations must, of necessity, be administered to humans with caution, even after they have been properly evaluated by toxicologic and pharmacologic studies in the lower animals. In the human being, it is necessary to employ cross-over experiments with placebos in order to eliminate the subjective bias of both the investigator and the subject. The placebo must produce the same side reactions as the test agent, without manifesting the analgesic activity of the preparation.

The use of animals removes the disadvantage of having to perform preliminary toxicologic investigations. Likewise their employment permits a larger number of analgesic agents to be screened within a relatively short period of time. Difficulties inherent in animal studies are indistinct response to pain stimuli, and reaction to other sensations simulating pain. The effect of the drug upon pain in the test animal must be transposed to clinical material in order to verify the relative activity of the preparation.

*Induction of pain*

The pain stimuli employed in analgesic screening techniques include the following: mechanical, chemical, electrical, or thermal. The earliest procedures used with the mechanical methods involved pinching of a rat tail and noting the manifestation of pain (4). The method was not sensitive enough for testing some of the mild analgesic agents. The trauma of the mechanical stimulus often prevented repeating the test and using the same animal. The lack of a precise endpoint for discerning pain rendered the method unsatisfactory. As a rule, the test methods have also involved the use of muscle ischemia (5) and visceral pain (6). These techniques result in severe pain which will usually not respond to the aspirin type of analgesic agent. Chemical induction of pain has been based upon local stimulation or production of swollen joints by injection of a chemical (7). It has been said that this type of reaction could detect aspirin analgesic activity. Both mechanically and chemically induced pain are stimuli fairly difficult to control and measure. The acceptable method for screening analgesic compounds at the present time is based upon the use of either electrical or thermal stimuli for the induction of pain. Of the two, thermal stimuli are the most widely used. Head-induced pain reactions are perhaps the simplest to control and standardize. In the popular radiant-heat method of Hardy, Wolff and Goodell (8), the apparatus administers a constant thermal stimuli to the blackened forehead of the human subject. Criticism directed against this widely accepted technique has

been based upon the fact that the pain is felt at certain critical temperatures and the drug may delay the arrival of this temperature by altering the vascular circulation. Nevertheless, it is the opinion of many investigators that the stimulus induced by the Hardy, Wolff and Goodell apparatus lends itself well to quantitative measurement. The method involves increasing the intensity of heat and determining the level at the time the subject notes pain. A modification of the method is based upon the principle that a constant heat stimulus should be used, and that the time required by the subject for detection of pain should be measured (9).

*Methods for study of pain response*

D'Amour and Smith adapted the radiant-heat principle of Hardy, Wolff and Goodell to the study of pain in rats (10). The endpoint employed was the tail-flip response to induced pain when the light of the apparatus was focused upon the tip of the rat tail. This method has since been applied to dogs with favorable results (11). A modification of the radiant-heat method has been developed for mice, whereby the animals are placed upon a hotplate, the surface temperature of which is accurately controlled. The end reaction is based upon the mice raising their paws and licking them. The time interval is noted (12). Various modifications of the D'Amour and Smith method have been employed.

After assessing the various procedures that have been used for the screening of compounds for analgesic activity, we employed a modification of the D'Amour-Smith method in our laboratory. In our hands this technique has given fairly reliable and predictable results (13). The method involves the use of the radiant-heat principle, whereby a light of constant intensity is focused by means of lenses and condensers upon the tip of the tail of a rat. Figure 1 depicts the apparatus timer, heat meter, and the groove into which the rat tail is placed. Animals weighing between 135 and 165 grams are usually employed. The duration of the pain stimulus is fifteen seconds, automatically controlled by a timer which activates the light when the timer is turned to the 15-second mark. The light is automatically turned off at the end of the 15-second period. In addition to the light-lens system, the apparatus consists of a constant voltage apparatus and volt meter. Any change in the voltage in the line is rectified by the constant

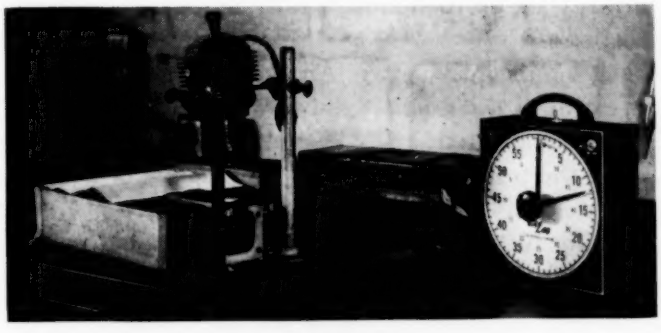

FIGURE 1

voltage apparatus, so that the light emits a constant amount of heat throughout the 15-second period. In addition to the apparatus described, a heat meter is placed in an area beneath the tip of the rat tail so that the light intensity can be measured and a specific heat reading maintained.

When all the conditions are properly standardized, the animal, prior to injection of the drug, is subjected to a control run whereby its tail is placed in the groove noted on the platform in Figure 1. The timer is set at the 15-second period; this automatically results in starting the radiant-heat system. During the 15-second period the beam is focused upon the tip of the tail. A rat failing to respond within the 15-second control period is not suitable for use in analgesic testing. The great majority will respond within a period of not more than nine to twelve seconds.

The material to be tested is measured on the basis of millimols per kilogram of body weight and is usually injected intraperitoneally. At least 10 animals are used for each dose level of the drug to be tested. The initial dose selected is one-fourth the toxic dose. After the injection has been completed the rat is tested at 20-minute intervals. A record is made of the number of animals that fail to respond during any of the testing periods; the number failing to show a reaction to the pain stimulus thus furnish a percentile estimate of the effective analgesia induced by the preparation being tested. This estimate is dependent upon the action of the rodents during the 15-second test period in which the light has been focused on the tail. Each rat being tested is submitted to four consecutive 20-minute observation periods after administration of the drug. If there is significant analgesia during the third of fourth period, testing may be continued in order to determine the duration of the analgesia.

In addition to the percentage of animals showing analgesic effects, other reactions are also recorded. These may include such disturbances as salivation, depression, central nervous system excitation, diarrhea, or lacrimation. Thus further clues as to possible pharmacologic activity of a drug are obtained. During the preliminary testing period, when toxicity alone is being determined, these undesirable side reactions are also looked for and recorded. The preliminary toxicologic studies are made in order to derive the initial dose of the analgesic.

*Criteria of analgesia*

We have arbitrarily set up the following criteria of significant analgesic effect. If only up to 30 per cent of the animals show analgesia, the compound is not considered as being significantly active. An analgesic effect of 1+ is recorded, if from 30 to 50 per cent of the animals show analgesia; 2+, if from 50 to 80 per cent of the animals are affected; and 3+, if at least 80 per cent are affected by the drug. If, during the testing procedure, 2+ analgesia or more is not obtained at a dosage level one-fourth that of the toxic level, then the compound is not considered suitable for further testing. However, if the analgesic effect is 80–100 per cent, with doses that do not result in toxicity, further investigation of the compound is warranted.

*Comparative analyses*

A comparative analysis of the activities of known analgesic compounds is presented in Figures 2–5. In Figure 6 a comparison of the activities of these compounds is made by plotting the logarithm of the dose against the logarithm of response for each of four preparations—morphine sulphate, codeine sulphate, methadon, and meperidine. As may be seen from the data presented in Figures 2–5, there was significant activity with all the preparations employed. However, only morphine resulted in 3+ analgesia in doses that were not toxic (Table 1). The toxicity in all cases was manifested by central nervous system depression or excitation or by other undesirable side effects that would preclude the efficacious use of these preparations. In animals, the dose-response relationships using morphine and codeine and the synthetic drugs, methadon and meperidine, were comparable to those observed clinically.

Based upon the data presented and on unpublished results with unknown compounds, we feel that the described modification of the D'Amour-Smith method, based upon the Hardy, Wolff and Goodell apparatus and technique, adequately measures the response of the animal to heat-induced pain. The data obtained on several standard compounds show a proper dose-response relationship, and appear to approximate those observed when the drugs are used clinically. The technique described appears to have fulfilled the prerequisites stated earlier in our discussion. First, the method for induction of the stimulus is simple, and the endpoint is readily detected. Secondly, the data indicate that the method is sensitive enough to establish a definite dose-response relationship. Thirdly, it

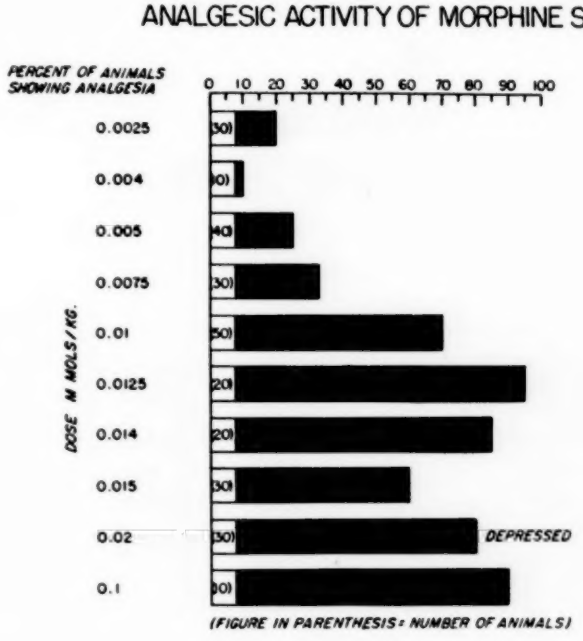


FIGURE 2

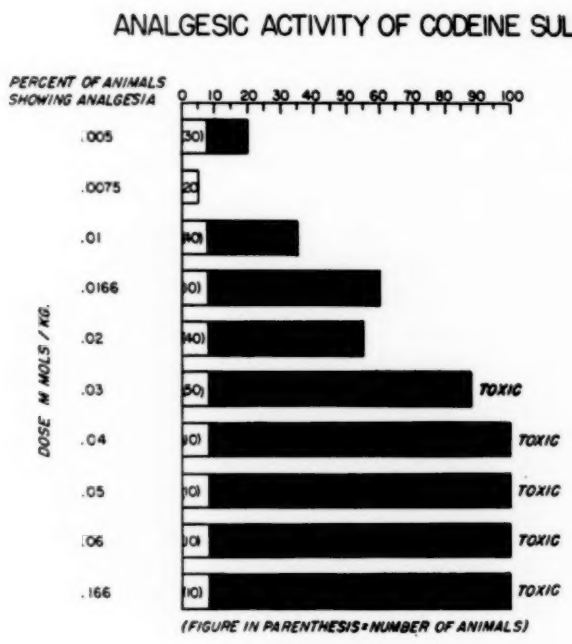


FIGURE 3

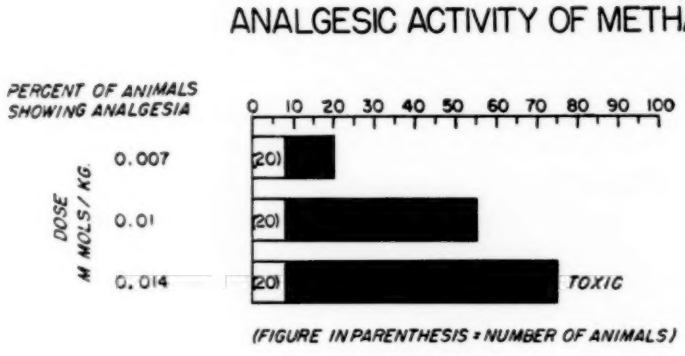


FIGURE 4

is sensitive enough to differentiate between the dose-effects of non-narcotic compounds. For example, with this procedure we were able to demonstrate that gentisic acid possesses adequate analgesic properties. We also showed that intraperitoneal administration of salicylates had no significant analgesic effect; upon oral administration, however, salicylates had significant (+) analgesic activity. It was suggested that perhaps after intraperitoneal injection salicylates are not metabolized into their analgesic components, so that definite analgesic effects cannot be detected, such as occur following oral administration. The fourth prerequisite for a testing procedure is that the results should show adequate correlation, with clinical findings. We feel that the dose-response relationships demonstrated with known analgesic compounds by our technique yielded

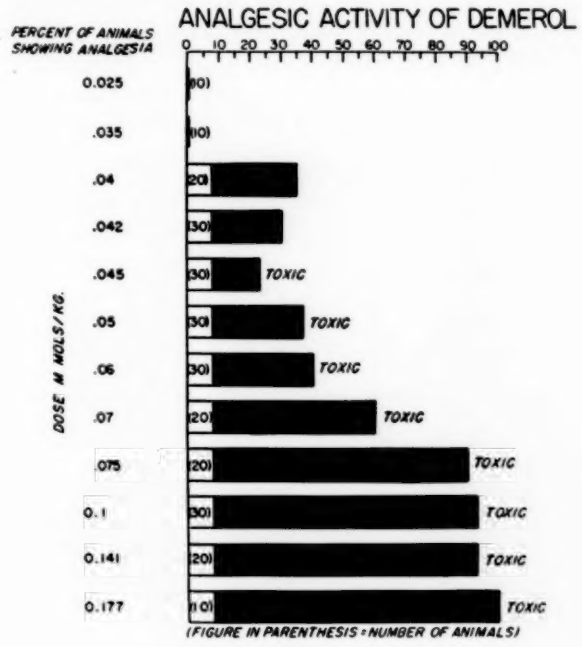


FIGURE 5

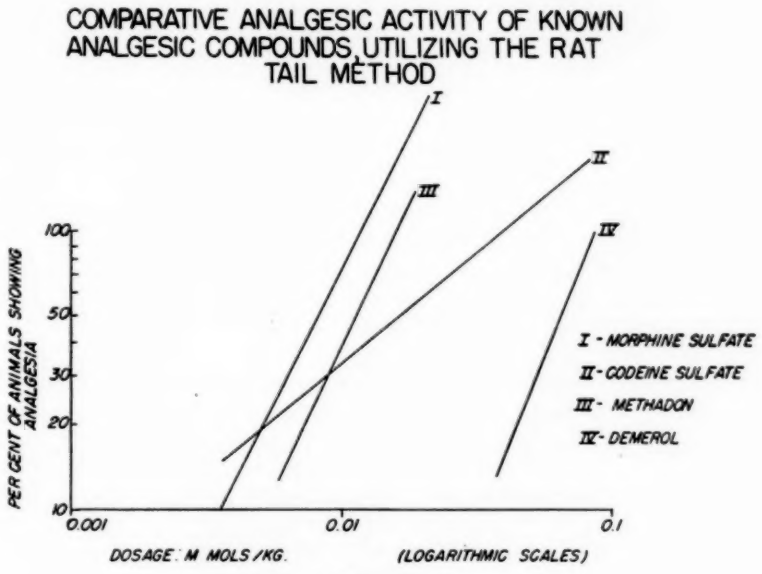


FIGURE 6

results which correlated well with observations on the same compounds during clinical use.

We have recently had the opportunity to evaluate three compounds clinically that had shown adequate analgesic properties when tested by the rat-tail method. The clinical analgesic data showed excellent correlation with the animal data,

TABLE 1

*Analgesic Activity of Standard Preparations as Measured by the Radiant Heat Rat-tail Method*

| Preparation | Dose in m mols./Kg. | No. of Animals | Percentage of Animals Showing Analgesia | Remarks |
|---|---|---|---|---|
| Morphine sulfate | 0.0025 | 30 | 20 | |
| | 0.004 | 10 | 10 | |
| | 0.005 | 40 | 25 | |
| | 0.0075 | 30 | 33 | |
| | 0.01 | 50 | 70 | |
| | 0.0125 | 20 | 95 | |
| | 0.014 | 20 | 85 | |
| | 0.015 | 30 | 60 | |
| | 0.02 | 30 | 80 | Depressed |
| | 0.1 | 10 | 90 | |
| Codeine sulfate | .005 | 30 | 20 | |
| | .0075 | 20 | 5 | |
| | .01 | 40 | 35 | |
| | .0166 | 10 | 60 | |
| | .02 | 40 | 55 | |
| | .03 | 50 | 88 | Toxic* |
| | .04 | 10 | 100 | Toxic* |
| | .05 | 10 | 100 | Toxic* |
| | .06 | 10 | 100 | Toxic* |
| | .166 | 10 | 100 | Toxic* |
| Methadone | 0.007 | 20 | 20 | |
| | 0.01 | 20 | 55 | |
| | 0.014 | 20 | 75 | Toxic |
| Merperidine | 0.025 | 10 | 0 | |
| | .035 | 10 | 0 | |
| | .04 | 20 | 35 | |
| | .042 | 30 | 30 | |
| | .045 | 30 | 23 | Toxic* |
| | .05 | 30 | 37 | Toxic* |
| | .06 | 30 | 40 | Toxic* |
| | .07 | 20 | 60 | Toxic* |
| | .075 | 20 | 90 | Toxic* |
| | 0.1 | 30 | 93 | Toxic* |
| | 0.141 | 20 | 93 | Toxic* |
| | 0.177 | 10 | 100 | Toxic* |

* Animals showed evidence of central nervous system depression or excitation.

with two of the preparations. The efficacy of the third drug did not approach that anticipated from the laboratory data.

## SUMMARY

A discussion of the factors involved in testing the analgesic properties of unknown chemical compounds is presented. The data indicate that the radiant-

heat rat-tail method is the most reliable testing procedure that may be used experimentally; methods involving mechanical, chemical and electrical stimuli are more difficult to control. Using a modification of the radiant-heat method, four standard compounds with known analgesic properties were evaluated and their effectiveness compared. From these data it is concluded that this analgesic testing procedure, which is essentially the D'Amour-Smith modification of the Hardy, Wolff and Goodell method, will yield reliable results regarding the correlation between the experimental effectiveness and the clinical effectiveness of the compound tested.

*Acknowledgment*

Appreciation is expressed to Lederle Laboratories Division of the American Cyanamid Company for research grants in support of investigation in the field of analgesia.

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# Obituaries

## LAURENCE E. HINES

The American Therapeutic Society and American Medicine sustained a great loss in the death of one of its members, Dr. Laurence E. Hines of Chicago, who passed away on May 13, 1955 at the age of 58. Dr. Hines was born in Livermore, Iowa, June 8, 1896. He did his college work at Creighton University, later transferring to Northwestern University of Chicago, where he graduated with a doctorate in medicine in 1919. His internship and residency were at Cook County Hospital, where he acquired an interest in clinical pathology and teaching that lasted throughout his entire medical life. In 1921 he married Miss Doris Zinn of Chicago. Six children were born of that marriage, Laurence E. Jr., John Richard, Dr. David W., Dr. James J., Miss Anne, and George A. The latter finished college this year. Dr. Hines joined the faculty at Northwestern University Medical School in 1923, his teaching at that time being in physical diagnosis and in cardiology. He was a member of the staff at St. Joseph's Hospital in Chicago, where he was chief of medicine for many years. He was also on the staff of Passavant Hospital in Chicago. He became professor of medicine at Northwestern University in 1947. He joined the American Therapeutic Society in 1944. He was a member of the Chicago Society of Internal Medicine and a member of the Executive Committee and past president of that society. He was also a Fellow of the American College of Physicians, and a member of the Institute of Medicine, American Heart Association, American Medical Association, Illinois State Medical Society, Chicago Medical Society, Chicago Pathological Society, Sigma Chi, and Nu Sigma Nu. He was an author of over forty articles. Four years before his death he suffered a stroke, which incapacitated him for a long period. He fought his way back and gradually began to resume the practice of medicine. He attended the last meeting of the American Therapeutic Society in St. Louis in November 1954. Larry was kindly, sincere, and hated pretense. He had just returned from a trip to Europe one month before he died.

THOMAS J. COOGAN, M.D.

## PAUL A. McNALLY

The Rev. Paul A. McNally, S.J. died March 4, 1955 of a heart attack. He was born in Philadelphia on October 14, 1890, the son of Charles A. and Martha Tully McNally. He received the A.B. degree in 1915 and the A.M. degree in 1917 from Woodstock College, Maryland. He studied at the University of Innsbruck, Austria, and received his Ph.D. from Fordham University in 1921. He earned the S.T.D. from the Gregorian University, Rome, in 1925. He studied at the University of California from 1926 to 1928.

Father McNally entered the Society of Jesus on August 12, 1908, and was ordained at Woodstock in June, 1923.

He served as professor of mathematics at Boston College from 1916 to 1919;

professor of higher mathematics and philosophy at Fordham, 1919–1921; assistant director, Georgetown Astronomical Observatory, 1925 to 1928, and as director, from 1928 to 1942.

In 1942, Father McNally was named vice-president of Georgetown University. Beginning in 1946, he served as regent and dean of the Medical School, and regent of the Dental and Nursing schools. Also in 1946 he was named the first director of the Georgetown University Medical Center, encompassing the new 400-bed Georgetown University Hospital which opened its doors in 1947.

Last year Father McNally was named chairman of the Physics department at Georgetown, a post he held at the time of his death.

Father McNally participated in solar eclipse expeditions to many parts of the globe. In 1936 he directed a Georgetown expedition to Fryeburg, Maine, and in 1936 he led a Georgetown-National Geographic party to Kustanai, U.S.S.R. He was a member of the National Geographic—U. S. Navy expedition to Canton Island in 1937, and of the National Geographic—National Bureau of Standards trip to Patos, Brazil, in 1940.

Father McNally was a trustee of the Hospital Service Agency, Group Hospitalization Inc., and honorary consultant to the Surgeons General of the U. S. Navy, Army and Public Health Service. He was a fellow of the American Association for the Advancement of Science and the Royal Astronomical Society of England. He was a member of the American Astronomical Society, Astronomical Society of the Pacific and Washington Academy of Sciences, and the Cosmos Club.

He wrote numerous articles and scientific papers on astronomical subjects.

Honorary degrees conferred on him were the Sc.D. from St. Joseph's College, Philadelphia, the LL.D. from the University of San Francisco, and the LL.D. from Georgetown.

ROBERT J. COFFEY, M.D.

## A. MORRIS GINSBERG

Dr. Ginsberg, an internist, practiced in Kansas City, Missouri from 1921 until his death on November 12, 1954, at the age of 59 years. His death was due to an acute heart attack which culminated five years of cardiac disability. He was widely known for his special interest in cardiovascular diseases, and served as a member of the Board of Directors of the Missouri Heart Association. He was also a past-president of the Greater Kansas City Heart Association.

Born in Kansas City in 1895, he received his Bachelor of Arts Degree from the University of Missouri in 1918 and his Doctorate of Medicine from the University of Pennsylvania in 1920. Following an internship in Philadelphia, he returned to his home city to enter the practice of internal medicine.

Dr. Ginsberg was an active member of many medical organizations and devoted much of his time and energy to these extracurricular activities. Among the special societies of which he was a member were the American College of Physicians, The American Therapeutic Society, The Endocrine Society, The

American Goiter Association, the Kansas City Anatomical Society, the Kansas City-Southwest Clinical Society, of which he was director of clinics in 1930 and 1931, and the American Society for Study of Arteriosclerosis.

In addition to an active practice in his special field, Dr. Ginsberg found time for medical teaching. He was affiliated with the University of Kansas School of Medicine as a clinical professor of medicine and in this capacity contributed greatly to the teaching program of that school.

Dr. Ginsberg was well-known and admired by his colleagues of the profession of Kansas City and the State of Missouri. He was always ready to assist in civic or welfare programs and gave freely of his time. As a fitting memorial to this physician, his friends are establishing a three-day seminar in diseases of the heart, to be held each year at the Menorah Medical Center with a guest speaker participating.

Perhaps no greater tribute can be given our departed colleague than that expressed at the time of his memorial service by Rabbi Meyerding, the spiritual leader of his congregation—"This integration of knowledge and spirituality was the motivating force of his life, governing all phases of his existence. It gave him firm belief in the integrity and individuality of each man. To those whom he treated he gave not only scientific knowledge but gentleness and an appreciation of the dignity of life."

We have lost a colleague and a friend; his patients have lost a physician, a counsellor and a friend. Our sympathies are extended to bereaved members of his family, his wife, a son and a daughter.

ALPHONSE MCMAHON, M.D.

## THE FIRST OSCAR B. HUNTER MEMORIAL AWARD IN THERAPEUTICS

Recipient: *Jonas E. Salk, M.D.*, Pittsburgh, Pa.

"Considerations in the Preparation and Use of Poliomyelitis Virus Vaccine"

Highlight of the fifty-sixth annual session of the American Therapeutic Society in Atlantic City was the first presentation of the Oscar B. Hunter Award in Therapeutics on June 4, 1955, to Dr. Jonas E. Salk. Dr. Salk gave an outline of the scientific facts leading the development of the currently used poliomyelitis vaccine and he traced his own interest in the development. Studies begun under Doctor Thomas Francis, Jr. at Ann Arbor, Michigan finally reached fruition, as announced April 12, 1955, by the Polio Foundation, in an effective vaccine, suitable for large-scale production. The facts regarding immune response to one or more doses of the vaccine were discussed and have since appeared in the Journal of the American Medical Association*. In addition to his major engrossment in research, Dr. Salk was found to be interested in tennis, golf, boating, fishing and classical music.

* *J.A.M.A.* **158:** 1239–1248 (Aug. 6) 1955.

# Abstracts of Current Literature

### RHEUMATOID ARTHRITIS AND POLYARTERITIS NODOSA

Ball. J. *Ann. Rheumat. Dis.* **13:** 277 (Dec.) 1954.

Evidence cited indicates that a small minority of rheumatoid arthritis patients, and a minority of patients with polyarteritis nodosa, show features of both syndromes. Clinical and necropsy findings in 5 patients illustrate this association. In 4 patients, polyarteritis nodosa appeared as a complication of rheumatoid arthritis. In 1 of these, it was an incidental finding, and in 3 a major contributory cause of death. None of these patients had received ACTH, and only 1 had received cortisone. In a fifth patient, a mild multiple arthritis of rheumatoid type and of three months' duration occurred in a patient with polyarteritis nodosa. It virtually disappeared during cortisone treatment of the polyarteritis. At necropsy the joints showed typical rheumatoid changes in the absence of articular arteritis. Although the association of polyarteritis nodosa with rheumatoid arthritis is rare, it is probably more than coincidental. The available evidence suggests that the distribution, frequency, and morphology of arterial lesions in rheumatoid arthritis are variable, and range from a mild indeterminate arteritis to classical polyarteritis nodosa.

### ACUTE PANCREATITIS SIMULATING MYOCARDIAL INFARCTION WITH CHARACTERISTIC ELECTROCARDIOGRAPHIC CHANGES

Bauerlein, T. C., and Stobbe, L. H. O. *Gastroenterology* **27:** 861 (Dec.) 1954.

In a case of acute pancreatitis severe electrocardiographic changes were judged to indicate a myocardial infarction localized to the anterior wall. The necropsy showed no lesion of the coronary arteries nor evidence of infarction, even on serial microscopic section. The patient died thirty hours after the electrocardiogram was obtained, so that it was believed that, had an infarction occurred, there would have been evidence of it. This case and others reported in the literature indicate that in acute pancreatitis the electrocardiographic changes of myocardial infarction of various degrees, stages, and locations can be simulated. The phenomenon has not been satisfactorily explained. The changes are not induced by shock nor altered states of the serum potassium content. That fat necrosis in the myocardium itself or some remote chemical effect of massive fat necrosis may produce the electrocardiographic changes is only conjecture.

### PRELIMINARY CLINICAL TRIALS WITH 9-ALPHA-FLUOROHYDROCORTISONE ACETATE IN RHEUMATOID ARTHRITIS

Boland, E. W., and Headley, N. E. *Ann. Rheumat. Dis.* **13:** 291 (Dec.) 1954.

9-Alpha-fluorohydrocortisone was administered as investigative therapy in 13 patients with active rheumatoid arthritis. A daily dosage of 3 to 8 mg. brought about rapid improvement in rheumatic manifestations. A comparison of maintenance dosages required for equivalent degrees of clinical control showed the halogenated derivative of hydrocortisone to be about ten times more potent in antirheumatic effectiveness than hydrocortisone free alcohol. However, with even the small daily amounts of the fluoro compound used, fluid retention developed in 12 of the 13 patients and was pronounced in some. This enhanced salt and water retaining property would seem to preclude the use of the substance for systemic therapy of rheumatoid arthritis.

PRIMARY MALIGNANCIES OF THE PAPILLA OF VATER. CASE REPORT AND REVIEW

Boleman, A. P., Jr., and Breslaw, L. *Gastroenterology* **27:** 869 (Dec.) 1954.

A review of 8 proved cases of carcinoma of the papilla of Vater seen at the Lenox Hill Hospital during the last twenty years, and a detailed case report of the latest patient, are presented. The average age was 63.4 years and the sex incidence was equally divided. The total duration of illness in 62.5 per cent of the cases was less than seven months. Although initial symptoms were varied, icterus was present in all patients prior to operation. Except for 2 who had previous cholecystectomies, and 1 patient who had chronic cholecystitis and cholelithiasis, the gallbladder was enlarged and palpable in accordance with Courvoisier's Law. X-ray studies of the gastrointestinal tract did not aid materially in either establishing or suspecting the diagnosis in 4 of the 5 patients. In all patients in whom stool studies for occult blood were performed, the results of guaiac determinations were positive. (From authors' summary.)

SENILE ECTROPION

Callahan, A. *Am. J. Ophth.* **38:** 787 (Dec.) 1954.

The types of senile ectropion and the surgical correction of each are discussed. Senile ectropion, a condition caused by senescent changes in the tissues of the lower eyelid, can be either the relaxed, the cicatricial, or the spastic variety. Senile spastic ectropion, which up to this time has not been described as a separate entity, requires a special kind of surgical correction. This type is usually unilateral, in contrast to the other two. It occurs when sagging of the lower lid due to senile relaxation coincides with spastic contraction of the orbicularis bands along the base of the lid. A case report illustrates the surgical procedure. The lid is shortened horizontally at the point of greatest relaxation, and part of the spastic orbicularis band is excised.

PERIPHERAL VASCULAR EFFECT OF CORTISONE IN RHEUMATOID ARTHRITIS, SCLERODERMA, AND OTHER RELATED CONDITIONS

Catchpole, B. N.; Jepson, R. P., and Kellgren, J. H. *Ann. Rheumat. Dis.* **13:** 302 (Dec.) 1954.

The peripheral vascular response to short courses of cortisone has been studied by a heat-flow technique in 22 patients suffering from rheumatoid disease, systemic sclerosis, and other related syndromes in which digital blood flow is reduced. During cortisone therapy there was a marked increase of digital blood flow in patients who experienced relief of joint pain and stiffness and in whom the reactive hyperemia time was less than five seconds. Results of the differential sheep cell agglutination test were positive in 14 of the 22 patients investigated, but the presence of a positive reaction was not specially associated with a good symptomatic or vascular response to cortisone. After the withdrawal of cortisone the digital blood flow usually fell to below pre-treatment levels. (From authors' summary.)

DILATATION JUGULAIRE NON OBSTRUCTIVE

Fiandra, O.; Cervino, J. M.; Navarro, A.; Maggiolo, J., and Mussio Fournier, J. C. *Presse méd.* **62:** 1826 (Dec. 25) 1954.

Observations are presented on the nonobstructive jugular-vein dilatation which occurs especially in menopausal women and almost always unilaterally on the left side. Clinically, the external jugular vein is dilated, the degree of dilatation varying with exertion, position, and psychic states. The symptoms are often poorly defined and may consist of a feeling of heaviness or pain radiating to the nape of the neck and the temporal region. The venous pressure measured by direct puncture shows normal values. Phlebography by injection of

a vein at the bend of the elbow shows the ascent of administered diodone by way of the internal jugular vein and the arrest of part of the substance at a bending in the brachiocephalic trunk; simultaneously there is dilatation of the jugular-subclavian confluence. A phlebogram illustrates the dilatation of the jugular vein in a 61-year-old man with symptoms of the male climacteric. Studies are in progress to determine the nature of this bending and the mechanism of its action, for it appears to be responsible for the jugular vein dilatation. Phlebograms are discussed and comparisons made with other jugular vein lesions.

THE AGEING SKIN

Freeman, J. T. *Pennsylvania M. J.* **57:** 1184 (Dec.) 1954.

Aging of the skin, which is the most evident of all the aging processes that occur in the body, may be brought on prematurely by environmental trauma. Protection of the skin from chronic low-grade overexposure to sunlight or to ultraviolet light is of great importance. The major distinctive problems of aging skin are disturbances in the growth pattern, which result in retrogressive functional and anatomic changes typical of atrophic aging tissue and in a unique group of proliferative disorders. The common dermatoses also frequently afflict the skin of the elderly. Dryness of the skin is common past the age of 35 years; the associated pruritus, when persistent, may indicate internal disease. The proliferative changes that occur require close medical attention. One in 3 of the older patients seen because of skin changes will have either a senile keratosis or a true epithelioma. Senile keratoses, which are real precancerous lesions, occur as red, scaly lesions of the exposed or aged skin. Destructive treatment is mandatory, and a diagnostic biopsy specimen should be secured. Epitheliomas appear as solid growths, with or without ulceration, and usually occur on exposed sites where aging is most advanced. Biopsy study is necessary to confirm the diagnosis and to determine the exact type. The more common basal-cell epithelioma is locally invasive but does not metastasize. The squamous-cell epithelioma is the serious malignant growth of the skin which may metastasize. Either type of epithelioma must be completely removed, by surgery, irradiation, or electrocoagulation. Seborrheic keratosis, another growth change typical of senile skin, rarely requires treatment other than for cosmetic reasons. The skin tag and the tiny angioma are also common and usually require no treatment.

PROVOCATIVE ASPECTS OF LIPOTROPIC THERAPY IN ANGINA PECTORIS

Goulder, N. E.; Kissane, R. W., and Bohl, R. W. *Am. Heart J.* **48:** 906 (Dec.) 1954.

Twenty-five patients with angina pectoris were evaluated before and during a course of lipotropic treatment with Granulestin, a soy lecithin preparation. Six subjects (24 per cent) appeared to improve on this program, even though very little objective change was evident in their cardiac status. Serum cholesterol levels decreased only slightly during treatment. Serum phospholipid concentrations definitely decreased, to effect an increase in the serum cholesterol/serum phospholipid ratio, a change which may be considered provocative in that no subject became worse and indeed some appeared better. These chemical changes were rather similar, both for the patients who manifested clinical improvement and for those who did not. In general these changes were maintained for three or four months and then regressed in irregular fashion. The tendency for serum cholesterol and serum phospholipid levels to return to pretreatment values despite continued therapy suggests that lipotropic agents, if apparently beneficial, should be used for only a few months at a time. The controversial issues of this study are discussed in the light of current theory. (From authors' summary.)

TESTOSTERONE IN CHRONIC TUBERCULOSIS

Griffith, R. L., and Linn, R. H. *Am. Rev. Tuberc.* **70:** 1020 (Dec.) 1954.

On the basis of observations on 17 male patients with chronic, active pulmonary tuberculosis who were given testosterone propionate intramuscularly, it is believed that this drug is worthy of trial in patients in whom weight gain is advisable. It can be used to supplement antimicrobial and surgical treatment. Fifteen of the 17 patients gained weight while receiving testosterone. After considering all factors in their cases, it is believed that 7 patients had good results, 3 had fair results, 5 had doubtful results, and 2 had poor results from the administration of the testosterone. The drug was well tolerated for long periods of time. Although some patients had good results from 25 mg. of testosterone propionate three times a week, it is suggested that 50 mg. be given three times a week for a trial period of one or two months, after which time it should be evident if weight gain will occur. (From authors' summary.)

LOCAL ANTIRHEUMATIC EFFECTIVENESS OF HIGHER ESTERS AND ANALOGUES OF HYDROCORTISONE

Hollander, J. L.; Brown, E. M.; Jessar, R. A.; Udell, L.; Smukler, N., and Bowie, M. A. *Ann. Rheumat. Dis.* **13:** 297 (Dec.) 1954.

Because the transitory palliative effect of intra-articular hydrocortisone acetate limits its practical value, several preparations of higher esters and some analogs of hydrocortisone were screened for prolonged local palliation. Hydrocortisone tertiary butyl acetate injected intra-articularly into the arthritic knees of 171 patients produced a greater and longer-lasting palliation than identical doses of hydrocortisone acetate in about 65 per cent of the patients. The effect and duration were no greater than those of hydrocortisone acetate in 35 per cent; in about 5 per cent hydrocortisone acetate was more effective. The 9-alpha-chloro and allo-dihydro analogs of hydrocortisone and the two higher esters, hydrocortisone caprylate and hydrocortisone benzoate, were assayed clinically along with these two substances in 17 patients with rheumatoid arthritis. The benefit was increased in a few cases by one or another of these substances, but none resulted in such consistent and prolonged improvement as hydrocortisone tertiary butyl acetate. Hydrocortisone benzoate proved impracticable because of synovial irritation. Allo-dihydro hydrocortisone was far less effective than hydrocortisone acetate. The reason for greater effectiveness in local palliation of arthritic symptoms of certain higher esters of hydrocortisone is unknown. Solubility is not the factor that determines length of action. By intra-articular injection in 37 cases of rheumatoid arthritis or osteoarthritis, 9-alpha-fluoro hydrocortisone acetate proved effective in a dosage of 3 to 5 mg. However, the degree of palliation and the duration of effect did not exceed the benefits with hydrocortisone acetate. When a dosage of 7.5 mg. was injected into one knee, or 5 mg. into each knee, marked edema of the leg or legs appeared and persisted for several days.

PAPILLARY TUMORS OF THE RECTUM

Kay, S., and Lee, H. C. *Virginia M. Month.* **81:** 573 (Dec.) 1954.

A review of recent literature concerning papillary tumors of the rectum serves as the background for a discussion of 6 cases. These tumors are found in an older age group than are the usual epithelial tumors of the rectum; all of the 6 patients were over 50 years of age. Malignant growths of the papillary adenoma type may be divided into those showing merely intramucosal changes and those characterized by penetration of neoplastic glands through the muscularis mucosa. As a group, the tumors are slow growing and late to metastasize. Those that show the severest anaplastic features histologically are more apt also to show microscopic evidence of invasion. Malignancy was ultimately diagnosed in all the

patients. Three had intramucosal carcinoma, and 3 had carcinoma of the infiltrating type. Abdominoperineal resection is indicated when the diagnosis is of infiltrative carcinoma. Histologic proof of invasiveness is necessary to justify radical procedures. Although local excision suffices for treatment of lesions confined to the mucous membrane, it is advisable to remove a ring of at least 2 cm. of normal mucosa around the tumor, and the patient must be observed for evidence of local recurrence.

THE TONSILS IN ADVANCED AGE

Kelemen, G. *Eye, Ear, Nose & Throat. Month.* **33:** 723 (Dec.) 1954.

The tonsil generally does not attain total involution during life time. In the curve of tonsillar involution, a plateau is found corresponding to the period between 45 and 65 years of age. Involution in the palatine tonsils is an atrophic process, cells decreasing in number and tissues in size without degeneration and without reverting to a functionally less active form before final disappearance. Scarcity of thromboses in veins and of perivascular infiltrations in the retrotonsillar space accounts, together with the diminishing mass of tonsillar tissue, for the infrequency of local reactions around the receding tonsil. At the same time, with parenchymal activity qualitatively unchanged, focal reactions appear in distant organs of the body as they do at any age. Operation on tonsils in persons of advanced age is a relatively unexacting procedure, to patient and surgeon. No greater operative risk is involved than in younger persons. There is no reason to withhold the benefits of this intervention because of advancing years. Preparation for undisturbed health in adult life calls frequently for removal of the tonsils in childhood; preparation for an undisturbed great age, in this era of rapidly increasing life-expectancy, should include the care of the tonsils, to which many difficulties can be ascribed in the later period, no less than in childhood. (From author's summary.)

SUPPRESSIVE AS COMPARED WITH ANALGESIC HORMONAL THERAPY IN PATIENTS WITH RHEUMATOID ARTHRITIS

Kinsell, L. W. *Ann. Rheumat. Dis.* **13:** 307 (Dec.) 1954.

Suppressive hormonal therapy was initiated in 54 patients with severe rheumatoid arthritis and carried on for a mean duration of sixteen months, but for as long as four years in some patients. Intensive therapy was not possible because of the development of psychosis, alarming gastrointestinal symptoms, or other undesirable effects in 17 patients. "Analgesic" hormonal therapy could be continued in 11 of them. The average maintenance dosage of ACTH was 6 units and the maximum, 40 units. The average maintenance dosage of cortisone was 40 mg., the minimum 25 mg., and the maximum maintenance dosage 150 mg. daily. The program for initial therapy of rheumatoid arthritis patients may be (a) hospitalization for ten days to two weeks, (b) intensive hormonal therapy to bring the red cell sedimentation rate to normal and to relieve pain and swelling in involved joints, (c) progressive decrease in dosage during the remaining period of hospitalization, to an amount sufficient to maintain clinical remission, and (d) instruction of the patient in the principles and practice of diet and medication. Most patients who are likely to undergo mental or gastrointestinal difficulties will do so during the initial intensive hormone administration. During hospitalization, therefore, it can be decided whether analgesic hormonal therapy or none should be employed. Estrogen and androgen, or both, may be of value in the program of treatment of menopausal women and elderly men. Intensive hormonal therapy is recommended because, with meticulous attention to diet and other adjuvant measures, the incidence of untoward side actions and the amount of hormone administered over a period of two years or more, will probably be no greater than with the lower dosages required in analgesic hormonal therapy. In addition, the net salvage rate among patients so treated will probably be greater.

THE CHOICE OF AMPUTATION IN SENILE GANGRENE

McGoey, P. F. *Canad. M. A. J.* **71:** 469 (Nov.) 1954.

A study of 105 patients 65 years of age or older with arteriosclerotic gangrene, seen during a ten-year period, revealed a high hospital mortality and a short average survival period following release from the hospital. Of the 51 patients who died in the hospital, major amputations had been performed on 31, minor amputations on 2, and no operation on 18. One year after the ten-year period, 27 of the 54 patients who had left the hospital were dead. Sixteen of these 27 had had major operations, 4 had had minor operations, and 7 had had no operation. The 27 patients still living included only 15 who had undergone major surgery; 10 of the survivors had had minor operations, and 2 had had no surgery. This indicates the need for careful evaluation of the individual patient and selection of the simplest possible amputation in cases of senile gangrene. In choosing the operation, it should be remembered that only rarely will a patient over 70 years of age be able to use an artificial leg successfully. Transmetatarsal amputation was performed on 12 of the patients, and encouraging results were obtained when a preliminary or simultaneous lumbar sympathectomy was performed. Satisfactory results were obtained in only 2 of the 6 patients who did not undergo sympathectomy, whereas the amputation was successful in 5 of the 6 patients who had sympathectomy.

MANAGEMENT OF INGUINAL HERNIAS IN THE AGED

Means, R. L. *Am. J. Surg.* **88:** 936 (Dec.) 1954.

Inguinal hernias occur in about 19 per cent of men over 50 years of age. An increasing incidence parallels the increasing age. In elderly surgical patients complications develop more easily, and are less easily mastered. Although the operative mortality is low in inguinal herniorrhaphies on elderly patients, the rate of aging appears to be increased by the procedure. A more conservative attitude is justified for asymptomatic indirect inguinal hernias in poor-risk patients. Symptomatic indirect hernias should be operated upon unless the risk is prohibitive. Asymptomatic direct hernias which are not of the unusual diverticular variety should not be operated upon in elderly poor-risk patients. For indirect hernias, surgical treatment should consist of high ligation of the sac and anatomic closure, without tension of the defect in the transversalis fascia. The defect should be closed snugly about the cord at the internal ring, even if the blood supply to the testicle is compromised. For combined indirect and direct hernias, anatomic repair is also recommended. If this is not feasible because of a large defect and unserviceable transversalis fascia, the Andrews or modified Halsted procedure is indicated, with or without resection of the spermatic cord. For sliding hernias in poor-risk patients reduction of the hernial mass, cord resection and complete closure of the internal ring may be the procedure of choice. (From author's summary and conclusions.)

THE EFFECT OF OESTROGENS ON SODIUM METABOLISM IN HYPERTENSIVE MEN

Ramsay, A. G.; Lewis, J. A., and Burt, N. S. *Canad. M. A. J.* **71:** 472 (Nov.) 1954.

A conjugated estrogenic (equine) preparation (Premarin) in daily doses of 0.625 or 1.25 mg. was given to 10 male patients with essential hypertension. The effect of this therapy on sodium metabolism was determined by means of long-term daily urinary sodium excretion studies and other ancillary determinations. Five patients failed to show any evidence of sodium retention. Five other patients showed a decrease in the urinary excretion of sodium. In 2 cases this was of no significance and in the other 3 cases the urinary excretion of sodium decreased from abnormally high values to values within the range of normal. The decrease was believed not to be indicative of sodium retention. This conclusion was

supported by the results of the ancillary determinations, namely, absence of increases in the body weight, blood pressure, plasma volume and heart size, and of electrocardiographic evidence of left ventricular strain. The changes induced by therapy in the serum values of sodium, cholesterol and phospholipid were not remarkable. Prolonged therapy with a conjugated estrogenic preparation in hypertensive men, in the dosage used, did not result in sodium retention. (From authors' summary and conclusions.)

KOMBINATIONSFORMEN VON LAENNECSCHER LEBERCIRRHOSE MIT DIABETES MELLITUS; IHRE BEZIEHUNGEN ZUR HÄMOCHROMATOSE

Regli, Von A. *Helvet. med. acta* **21:** 535 (Dec.) 1954.

Tests carried out on pathologic sections at the Pathology Institute of Zurich University indicate that diabetes mellitus and Laennec's cirrhosis occur together and with greater frequency than might be expected. Twenty-nine instances were found among 12,069 sections. The 2.4 per thousand frequency of the combination exceeded by three times the probability of these diseases occurring together by chance. Diabetes mellitus appeared to have preceded the cirrhosis in 18 instances. In 2 cases, the cirrhosis may have resulted from homologous serum jaundice. In 7 cases, cirrhosis and diabetes were diagnosed simultaneously. In them it was assumed that the cirrhosis occurred prior to the diabetes and they were classified with 4 patients in whom cirrhosis occurred first. These latter cases, particularly, in their clinical course, resembled hemochromatosis, a disease in which diabetes mellitus generally occurs after the onset of cirrhosis of the liver. It could not be stated that these cases represent an incomplete form of hemochromatosis, because specific pathologic and anatomic data indicating a difference between these cases of cirrhosis and those of ordinary Laennec's cirrhosis were lacking. Neither could uniform data be derived from a postmortem examination of the pancreas.

SERIAL ARTERIOGRAPHY IN ATHEROSCLEROSIS

Willis, G. C.; Light, A. W., and Gow, W. S. *Canad. M. A. J.* **71:** 562 (Dec.) 1954.

A study of patients with atherosclerosis by serial angiography is described, and therapy in atherosclerosis is discussed. Serial arteriography of the femoral and popliteal arteries in men from 55 to 77 years of age with symptoms of atherosclerosis revealed intimal plaques in 16 of the patients. On re-examination two to six months later, none of 6 patients used as controls had improved; the condition of 3 had deteriorated; 3 remained unchanged. Six of the 10 patients treated with 500 mg. of ascorbic acid three times a day orally had improved; in 3, the plaques had enlarged, and the condition of 1 patient remained unchanged. No new plaques were seen to develop during the period of observation. Regression and progression of plaques were observed to occur over relatively short periods of time; they were never coexistent. The fact that not all of the plaques in a given individual showed progression indicates the importance of local rather than systemic etiologic factors. Only in some of the patients was there a correlation between the arteriographic changes and the signs and symptoms. The plasma cholesterol levels were not observed to fluctuate in relation to the changes in the plaques. Studies of the pathogenesis of atherosclerosis in animals provides a rationale for ascorbic-acid therapy. The results of this study of ascorbic-acid therapy in human atherosclerosis as followed by serial angiography are encouraging. Serial angiography can be used to assess therapy.

FOOD USAGE AND FOOD HABITS OF OLDER WORKERS

Young, C. M.; Streib, G. F., and Greer, B. J. *A. M. A. Arch. Indust. Hyg.* **10:** 501 (Dec.) 1954.

Twenty-four-hour records were collected on the food intake of 1,640 64-year-old employees from 230 companies in 21 major indistrial and occupational categories. The records

were tabulated in terms of the number of servings of certain foods used per day, and the adequacy of the diets was evaluated. An insufficient consumption of milk was one of the most important deficiencies in the diets of these older employees; 22 per cent used no milk, and 62 per cent failed to meet the conservative standard of adequacy of two cups per day. Forty per cent of the men used less than 65 Gm. of protein per day, and 51 per cent of the women used less than 55 Gm. per day. Fifty-eight per cent of the men and 70 per cent of the women used one or more servings per day of fruits or vegetables high in ascorbic acid. Only 34 per cent of the men and 43 per cent of the women used one or more servings per day of fruits or vegetables high in carotene (vitamin A value). Ten per cent of the men and 7 per cent of the women received an over-all dietary rating of "adequate." The adequacy of the diet was found to increase with the educational level and, to a lesser extent, with the income level. A definite positive relationship existed between the use of important protective foods and the educational level. Investigation of food habits showed that only 3 per cent of the men and 5 per cent of the women missed any meal during the day of recording. Two-thirds of the workers ate between meals. The food habits and the adequacy of the diets of persons still actively employed at 64 years of age were found to be equal or superior to those of younger workers previously studied. This survey shows that there is still a need to encourage the use by industrial workers of milk and its products, and fruits and vegetables, particularly citrus fruits, tomatoes, and green, leafy and yellow vegetables.

## BOOKS RECEIVED

Receipt of the following books for review is hereby acknowledged. We are glad to publish complete listings of all new books received but such listing must be regarded as a sufficient return for the courtesy of the sender. Books of particular interest will be reviewed in full, as time and space permit. Additional information in regard to all listed books will be gladly furnished upon request.

HIGH BLOOD PRESSURE. By George White Pickering, M.D., Professor of Medicine, University of London, Physician to St. Mary's Hospital, London. Cloth. $9.50. Pp. 547. 106 illustrations. Grune & Stratton, Inc., New York, 1955.

MENTAL HYGIENE IN PUBLIC HEALTH. By Paul V. Lemkau, M.D., Professor of Public Health Administration, Division of Mental Hygiene, Johns Hopkins University, Baltimore. Cloth. $8.00. Pp. 486. McGraw-Hill Book Co., Inc., New York, 1955.

OLD AGE IN THE MODERN WORLD. Report of the Third Congress of the International Association of Gerontology, London, 1954. Cloth. $8.00. Pp. 647. E. &. S. Livingstone, Ltd., London, and The Williams & Wilkins Co., Baltimore, 1955.

EARNING OPPORTUNITIES FOR OLDER WORKERS. Edited by Wilma Donahue. Cloth. $4.50. Pp. 277. University of Michigan Press, Ann Arbor, 1955.

AGING AND RETIREMENT. Edited by Irving L. Webber. Paper. $2.00. Pp. 142. University of Florida Press, Gainesville, 1955.

OLD PEOPLE IN A MODERN AUSTRALIAN COMMUNITY. By Bertram Hutchinson, Ph.D. Cloth. $4.75. Pp. 180. Melbourne University Press; New York, Cambridge University Press, 1954.

MEDICAL PROBLEMS OF OLD AGE. By A. N. Exton-Smith, M.D., Physician, Whittington Hospital, London. Cloth. $7.00. Pp. 331. John Wright & Sons Ltd., Bristol, and The Williams & Wilkins Co., Baltimore, 1955.

THE FORESEEABLE FUTURE. By Sir George Thomson, Nobel Laureate, Master of Corpus Christi College, Cambridge. Cloth. $2.50. Pp. 166. Cambridge University Press, New York, 1955.

Books for review should be sent to Edward Henderson, M.D., Editor-in-Chief, 236 Midland Avenue, Montclair, New Jersey.